Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

DEPARTAMENTO DE INGENIERÍA QUÍMICA Y TECNOLOGÍA DEL MEDIO AMBIENTE

TESIS DOCTORAL:

INNOVATIVE ALGAL-BACTERIAL PROCESSES FOR WASTEWATER TREATMENT: A FURTHER STEP TOWARDS FULL SCALE IMPLEMENTATION

Presentada por Esther Posadas Olmos para optar al grado de doctora por la Universidad de Valladolid

Dirigida por:

Raúl Muñoz Torre
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PROCESOS INNOVADORES DE TRATAMIENTO DE AGUAS RESIDUALES MEDIANTE SISTEMAS ALGAS-BACTERIAS: UN PASO HACIA SU APLICACIÓN A ESCALA REAL

Presentada por Esther Posadas Olmos para optar al grado de doctora por la Universidad de Valladolid

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Memoria para optar al grado de Doctor, con **Mención Doctor Internacional**, presentada por la Ingeniera Química:

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Certifican que:

ESTHER POSADAS OLMOS ha realizado bajo su dirección el trabajo "*Innovative algal-bacterial processes for wastewater treatment: a further step towards full scale implementation*", en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente en la Escuela de Ingenierías Industriales de la Universidad de Valladolid. Considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresan su conformidad con dicha presentación.

Valladolid, _____de _____2016

Fdo. Raúl Muñoz Torre Fdo. Pedro A. García-Encina

Reunido el tribunal que ha juzgado la Tesis Doctoral Titulada "*Innovative algal-bacterial processes for wastewater treatment: a further step towards full scale implementation*" presentada por la Ingeniera Química Esther Posadas Olmos y en su cumplimiento con lo establecido por el Real Decreto 99/2011 de 28 de enero de 2011 acuerda conceder por_____ la calificación de_____.

Valladolid, _____de _____2016

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Resumen

Las tecnologías convencionales de tratamiento de aguas residuales (TAR) como fangos activos o procesos anaerobios conllevan altos costes de operación por la necesidad de aireación para la oxidación de materia orgánica y/o NH_4^+ o de aumentar la eliminación de nutrientes de las aguas para poder ser vertidas al medio, respectivamente. En este contexto, los procesos de TAR mediante sistemas simbióticos algas-bacterias constituyen una alternativa eficiente y medioambientalmente más sostenible a sus homólogos biológicos. Gracias a esta interacción simbiótica, el carbono orgánico de las aguas residuales es oxidado por los microorganismos heterótrofos a CO_2, el cual puede ser asimilado en forma de biomasa por las microalgas durante la fotosíntesis (en presencia de luz), junto con el carbono inorgánico y los nutrientes contenidos en las aguas residuales (nitrógeno y fósforo, principalmente). A su vez, el oxígeno producido durante la fotosíntesis es suficiente para que los microorganismos heterótrofos y autótrofos puedan oxidar la materia orgánica y el NH_4^+ de las aguas residuales, respectivamente, lo que representa un ahorro considerable en comparación con los sistemas tradicionales de aireación mecánica. Además, la biomasa generada durante este proceso tiene un valor añadido como materia prima para la producción de biocombustible y/o biofertilizante. Esta biotecnología permite integrar el tratamiento de aguas residuales y la limpieza de biogás o el tratamiento de gases de combustión (aprovechando el CO_2 de estos gases para crecimiento algal y así obtener una mayor eliminación de nutrientes de las aguas residuales). Sin embargo, para poder aplicar esta tecnología en las estaciones depuradoras de aguas residuales (EDARs) y asegurar su viabilidad y sostenibilidad es necesario que se lleven a cabo previamente algunas mejoras y optimización del proceso. Además, algunas de las limitaciones técnicas (como el alto coste de cosechado de la biomasa algal o los altos tiempos de residencia del agua residual requeridos para su tratamiento) deben de ser superadas. De este modo, esta tesis se centró tanto en la determinación del potencial como en la superación de las limitaciones que presenta el TAR en sistemas algas-bacterias como paso previo a su aplicación a escala real.

En el **Capítulo 1** se presenta el estado del arte de la biotecnología algas-bacterias para TAR, mientras que en el **Capítulo 2** se recogen los objetivos de la tesis y su desarrollo.

Debido a la alta variabilidad en la composición de las aguas residuales es necesario determinar las posibles limitaciones de las mismas para ser tratadas de manera óptima por sistemas algas-bacterias. Acorde a esta necesidad, en el **Capítulo 3** se estudia la biodegradabilidad por sistemas algas-bacterias en sistemas discontinuos y cerrados de cinco aguas residuales

agroindustriales: agua residual de procesado de patata (PW), de piscifactoría (FW), de producción de piensos de animales (MW), de café (CW) y de levaduras (YW). Se eligieron estas aguas residuales, en primer lugar, por pertenecer a agroindustrias características de la región (Castilla-León) y por sus altas concentraciones de carbono y nutrientes. También se eligieron éstas por tener composiciones muy distintas entre ellas, lo que proporciona un amplio abanico de posibilidades para determinar la posible aplicación y limitaciones de sistemas algas-bacterias aplicados a diferentes tipos de aguas residuales. Así, se concluyó que las altas concentraciones de NH_3 (≈100 mg $N\text{-}NH_4^+$ L^{-1} a pH 8) y los altos pHs (≥10) pueden inhibir la actividad del consorcio algal-bacteriano, por lo que para el tratamiento fotosintético óptimo de algunas aguas residuales sería necesario diluir previamente el influente a tratar y/o controlar el pH (normalmente con adición de CO_2). También se determinó que la relación óptima de composición C/N/P (carbono/nitrógeno/fósforo) en las aguas residuales para ser tratadas por esta biotecnología debe ser 100/18/2 (g/g/g), siendo el carbono orgánico biodegradable el principal limitante para obtener una mayor depuración de nutrientes.

Los altos costes de cosechado de biomasa algal en sistemas convencionales (normalmente sistemas de lagunaje en los que la biomasa se encuentra en suspensión) conllevan la necesidad de desarrollar sistemas más efectivos de recogida de la misma. En este contexto, en el **Capítulo 4** se estudia la eliminación de C, N y P de digestato y agua residual doméstica primaria en dos biorreactores abiertos de 31 L y 0.5 m^2 de superficie de cultivo en los que la biomasa crece adherida a las paredes del mismo (biopelícula - crecimiento inmovilizado). Uno de los biorreactores se operó con un período de luz:oscuridad de 16:8 horas con una radiación fotosintéticamente activa de 88±16 µmol m^{-2} s^{-1}, mientras que el otro biorreactor no se iluminó, comparándose en este sentido la diferencia entre un reactor de biopelícula algas-bacterias con uno de biopelícula de bacterias únicamente. El funcionamiento de los biorreactores sólo fue efectivo durante el tratamiento de agua residual primaria doméstica como consecuencia de la limitación por carbono en el digestato. Así, los resultados mostraron una eliminación similar de carbono total (orgánico e inorgánico, ≥80%) en ambos sistemas e independiente del tiempo hidráulico de residencia (THR) (3 – 10 días). En el fotobiorreactor iluminado, este carbono se eliminó por stripping y asimilación como biomasa algal, mientras que la alta actividad nitrificante en el sistema no iluminado disminuyó el pH hasta valores de 5.8, siendo la eliminación por stripping a estos pHs el principal mecanismo de eliminación de carbono. Por otro lado, a pesar de que todo el NH_4^+ fue oxidado en ambos sistemas independientemente del THR, la eliminación de nitrógeno fue dos veces superior en el sistema iluminado (principalmente por asimilación como biomasa algal y stripping), mientras que el

fósforo sólo fue eliminado de forma efectiva mediante su asimilación en forma de biomasa algal en el sistema iluminado. Así, las mayores eficiencias de eliminación de C, N y P (91±3%, 70±8% y 85±9%, respectivamente) se registraron para un THR de 10 días en el sistema iluminado. Sin embargo, la alta tasa de evaporación en este sistema (0.5-6.7 L m^{-2} d^{-1}) contribuyó al deterioro de la calidad del efluente. En este biorreactor (únicamente en el sistema iluminado porque en el sistema sin luz el crecimiento de biomasa fue despreciable) el cosechado de biomasa algal mediante raspado mecánico de la superficie fue efectivo y para secar la biomasa no fue necesario centrifugar el efluente. Sin embargo, la alta eliminación de C y N por stripping conllevó una baja productividad de biomasa (máx. 3.1 g m^{-2} d^{-1}).

En el **Capítulo 5**, con el objetivo de aumentar la productividad en fotobiorreactores de biopelícula y evitar tanto la eliminación de C y N por stripping así como las altas tasas de evaporación, se evaluó comparativamente el funcionamiento de un fotobiorreactor de biopelícula cerrado (tubular) y otro abierto. En ambos fotobiorreactores, con un volumen total de 31 L y 0.5 m^2 de superficie de cultivo, se trató agua residual doméstica primaria con un THR entre 5 y 10 d bajo un período de luz:oscuridad de 16:8 horas a una radiación fotosintéticamente activa de 74±3 µmol m^{-2} s^{-1}. Los resultados mostraron una eliminación de carbono orgánico similar en ambos sistemas (63-97%). Sin embargo, la eliminación de carbono inorgánico y nutrientes fue siempre más efectiva en el sistema abierto (sobre todo en cuanto a eliminación de P mediante su asimilación en forma de biomasa algal). Como consecuencia del pequeño diámetro de los tubos (1 cm) en el fotobiorreactor tubular, el excesivo crecimiento de biomasa algal en las primeras fases de trabajo provocó el colapso de los mismos, lo que conllevó una menor efectividad en el tratamiento del agua residual. De manera similar al capítulo 4, el tratamiento más efectivo del agua residual se registró para un THR de 10 d en el sistema abierto, con eliminaciones de C, N y P de 89±2%, 92±5% y 96±2%, respectivamente. Los mecanismos de eliminación de C, N y P fueron también los mismos que los registrados en el capítulo anterior. Durante esta experimentación también se concluyó que la composición de la biomasa algal fue bastante similar independientemente del tipo de fotobiorreactor y de las condiciones de operación (C: 43.1±2.3%, N: 7.6±0.7% y P: 0.9% para el sistema cerrado y C: 44.8±1.5%, N: 8.5±0.6% y P: 1.0±0.2% para el sistema abierto). Por otro lado, también se estudió la evolución de la población de microalgas en ambos biorreactores dependiendo de las condiciones de operación con el objetivo de determinar las especies de microalgas más efectivas para depurar el agua residual. Los resultados mostraron como en el sistema cerrado la diversidad de especies fue menor que en el abierto como consecuencia de su menor exposición al ambiente. En ambos sistemas, microalgas del género *Phormidium*, *Scenedesmus*

y *Chlorella* fueron identificadas, lo que indicó su resistencia a la contaminación y su idoneidad para el tratamiento de aguas residuales.

Los resultados obtenidos en los capítulos 4 y 5 mostraron la dificultad del posible escalado de los fotobiorreactores de biopelícula. En este escenario, el escalado de fotobiorreactores abiertos convencionales de crecimiento de biomasa algal en suspensión (en su modalidad high rate algal ponds (HRAPs)) cuenta actualmente con un mayor número de estudios. Sin embargo, son varias las limitaciones que se tienen que superar para su aplicación final en EDARs e industrias. Por ello, en el **Capítulo 6** se operó un HRAP de 180 L en exterior (Valladolid, España) desde abril hasta octubre con el objetivo de tratar agua de piscifactoría (que después sería combinada con agua residual urbana primaria por su limitación en nitrógeno). Uno de los objetivos de este trabajo fue estudiar la influencia de las condiciones ambientales de la región (caracterizada por veranos muy calurosos y secos) en la calidad final del efluente tratado. Así, las eliminaciones de Demanda Química de Oxígeno (DQO), nitrógeno Kjeldahl total (NKT) y fósforo total (PT) máximas para el agua de piscifactoría fueron de 77±9%, 83±10% y 94±6%, respectivamente, para un THR de 10 d. El C y N de las aguas residuales fueron eliminados por asimilación en forma de biomasa algal (52±12% y 74±22%, respectivamente) y volatilización, siendo el fósforo eliminado únicamente por asimilación como biomasa (69±23%). Debido a las altas eliminaciones de carbono por mecanismos abióticos y las bajas cargas alimentadas, la productividad en el sistema fue baja (máx. 5 g m^{-2} d^{-1}). El cosechado de la biomasa del efluente se realizó en un sedimentador de 8 L con una eficacia de 82±18%, lo que conllevó una concentración de sólidos en suspensión en el efluente final inferior a la establecida por la normativa Europea de descarga (35 mg sólidos suspendidos totales (SST) L^{-1}). Este método de eliminación de biomasa algal representa una alternativa muy económica en comparación con técnicas físico-químicas o mecánicas como centrifugación o filtración. La limitación principal del sistema fueron las altas tasas de evaporación, que alcanzaron máximos de 15 L m^{-2} d^{-1} a finales de verano. Sin embargo, y a pesar de la necesidad de seguir trabajando en la optimización de HRAPs para disminuir esta evaporación, el escalado de este sistema con una menor turbulencia (mediante el correcto dimensionamiento del motor) reduciría este valor considerablemente.

La efectividad en el TAR de los sistemas algas-bacterias mostrada en los capítulos anteriores permitió dar un paso más e integrar con este tratamiento del agua la limpieza del biogás obtenido en las EDARs para poder ser inyectado en las redes de gas natural o usado como combustible en automoción. En este escenario, en el **Capítulo 7** se estudió la integración del tratamiento de agua residual con biogás en un fotobiorreactor de 180 L interconectado a una

columna de absorción de biogás de 2.5 L mediante recirculación del efluente líquido con un período luz:oscuridad de 16:8 h y a una radiación fotosintéticamente activa de 104±25 µmol m^{-2} s^{-1}. En el **Capítulo 7.1** se trató digestato de EDAR diluido con el objetivo, en primer lugar, de evitar la inhibición de las microalgas por las altas concentraciones de amonio (446±101 mg N-NH_4^+ L^{-1}) y en segundo lugar, para poder trabajar en condiciones de limitación de nutrientes y promover la acumulación de lípidos en las microalgas. El sistema se operó a 7 d de THR, independientemente de la dilución del digestato (30 o 70 veces). El biogás sintético alimentado que se usó contenía únicamente CO_2 (30%) y N_2 (70%). Los resultados mostraron una eliminación máxima de CO_2 del 99%, conjuntamente con una eliminación completa de nitrógeno y del 82% de fósforo del digestato diluido 70 veces. Sin embargo, en estas condiciones de operación la concentración de O_2 en el biogás tratado fue alrededor del 20%, lo que no sería apto en ningún caso para ser inyectado en la red (máximo valor permitido de 0.3% según la legislación española). Por otro lado, el contenido en lípidos de la biomasa algal recogida fue muy bajo (2.9-11.2%) incluso en condiciones extremas de limitación de nitrógeno, lo que indicó que esta estrategia de operación no es adecuada para producción de biodiesel. La eficiencia de recogida de biomasa algal en el HRAP en un sedimentador de 8 L fue del 95%, corroborando la efectividad mostrada en el capítulo 6. Finalmente, también se estudió la evolución de la población de microalgas, mostrándose una alta variabilidad y diversidad, sin que predominara únicamente un tipo de microalga a lo largo de toda la operación. En el **Capítulo 7.2** se trabajó en este mismo sistema y en las mismas condiciones de iluminación para tratar digestato de vinaza digerida y vinaza sin digerir (ambos efluentes diluidos). En este caso, la composición del biogás sintético fue de CO_2 (29.5%), H_2S (0.5%) y CH_4 (70%). Durante esta experimentación el objetivo principal fue disminuir la cantidad de oxígeno en el biogás purificado. En estas condiciones, se consiguió un valor mínimo de O_2 en el biogás depurado del 0.7±0.2% cuando la vinaza sin digerir y sin diluir se alimentó directamente en la columna de absorción con el fin de promover el consumo microbiano del O_2 disuelto para la oxidación de materia orgánica (lo que redujo la desorción del mismo al biogás tratado) para un THR de 7 d. En estas condiciones, la eliminación de CO_2 y H_2S del biogás sintético fue del 72±1% y del 100±0%, respectivamente. Sin embargo, al tratarse de un sistema abierto tuvo lugar la desorción del N_2 disuelto en el caldo de cultivo, lo que redujo la pureza final del biogás tratado a un contenido en CH_4 (81±2%) inferior a la requerida por la ley (≥95%). A su vez, la eliminación de C, N y P de la vinaza cruda diluida fue del 72±4%, 74±3% y 78±5%, respectivamente. La máxima productividad de biomasa que se alcanzó fue de 16.9±0.7 g m^{-2} d^{-1}, que coincidió con el período de alimentación de vinaza cruda diluida, mientras que la eliminación por sedimentación de la biomasa algal del efluente tratado fue efectiva en todas las condiciones

de operación (98.6±0.5%). Finalmente, el estudio de las dinámicas de población de microalgas y bacterias reveló en ambos casos una alta diversidad de especies en cualquiera de las condiciones de operación evaluadas.

En el **Capítulo 8** se integró también el tratamiento de agua residual y de gases de combustión, lo que teóricamente aumentaría la eliminación de nutrientes del agua residual por aumento en la producción de biomasa. Esta experimentación se realizó en exterior (Almería, España) en fotobiorreactores de 700, 800 y 850 L de volumen total desde julio hasta diciembre y se estudió la influencia del pH (7, 8 , 9) y de la fuente de CO_2 para el control de pH (CO_2 puro o gas de combustión) en la efectividad de tratamiento de agua residual doméstica primaria (DQO, nitrógeno total (NT), PT y *Escherichia coli*), en la composición de la biomasa algas-bacterias y en la productividad. Los sistemas se operaron a un THR entre 2.7 y 6.7 d, lo que supuso una disminución considerable respecto a las experimentaciones de los capítulos anteriores. Las eliminaciones medias de DQO, NT, PT y *E. coli* a lo largo de los 6 meses de operación fueron de 84±7%, 79±14%, 57±12% y 93±7%, respectivamente. En el rango de pH de trabajo (de 7 a 9) no se registró variabilidad en ninguno de los parámetros evaluados. Sin embargo, la adición de CO_2 de gas de combustión en vez de CO_2 puro mostró una ligera mejora en el tratamiento del agua residual (posiblemente como consecuencia de una mejor eliminación de oxígeno al estar más tiempo la válvula de CO_2 abierta para poder controlar el pH de manera adecuada). También se comparó el funcionamiento de los fotobiorreactores con y sin adición de CO_2, concluyéndose que en ninguno de los casos el CO_2 aportado por el gas de combustión aumentó la eliminación de nutrientes o productividad de biomasa algal como consecuencia de su alta tasa de eliminación por stripping. La productividad de biomasa se mantuvo entre 17±1 g m^{-2} d^{-1} en julio y 4±0 g m^{-2} d^{-1} en diciembre. El mayor contenido de C, N y P (64.8%, 12.6% y 2.4% respectivamente) en la biomasa se registró con adición de CO_2 de gas de combustión. Por otro lado, la composición en proteínas de esta biomasa fue bastante constante a lo largo de toda la operación (38.2±3.3%), a pesar de que la composición en lípidos y carbohidratos fue bastante variable (desde 5.8% a 23.0% y de 38.0% a 61.2%, respectivamente). Finalmente, la medida del máximo rendimiento cuántico de la biomasa (Fv/Fm) mostró cómo las altas radiaciones solares en verano (hasta 900 W m^{-2}) disminuyeron la actividad de las microalgas.

La integración de HRAPs en EDARs ha sido propuesta como tratamiento secundario y terciario. Por otro lado, también han sido varias las aplicaciones de la biomasa algal que se han propuesto para revalorizar la biomasa generada durante el proceso de tratamiento. Sin embargo, es necesario definir las etapas previas y posteriores necesarias al HRAP en EDARs para cumplir con los límites de descarga de las aguas residuales. A su vez, en todas las

experimentaciones realizadas con HRAPs tampoco se ha considerado el coste y manejo de los biosólidos durante su revalorización. En este contexto, en el **Capítulo 9** se tuvieron en cuenta estas limitaciones y se llevó a cabo un estudio de la implantación de HRAPs en dos configuraciones distintas de EDARs para una población de 2000 habitantes. En la primera configuración, el agua cruda residual es sometida a tratamiento primario previamente al sistema de algas y bacterias y la biomasa generada en el HRAP se separa por sedimentación y se digiere de manera anaerobia. En la segunda configuración, se lleva a cabo un pretratamiento del agua residual para eliminación de arena únicamente y la biomasa producida en el HRAP se separa en un sedimentador y se seca primero en un filtro prensa y después en un secadero solar. A pesar de la eficiente producción de energía durante la digestión anaerobia (10.7 € por habitante al año), el manejo del digestato producido, así como su almacenamiento y transporte para utilizar en los cultivos cuando es necesario, conllevaría elevados costes (32.5 € por habitante al año) y la implementación de esta biotecnología para pequeñas comunidades representaría un coste prohibitivo. Sin embargo, el secado de biomasa algal en invernadero permitiría almacenar durante un año los biosólidos y utilizarlos en los cultivos cuando fuera necesario y en estado sólido, reduciendo así hasta en 13 veces los costes en transporte.

Finalmente, todas las conclusiones de los trabajos realizados y las propuestas de investigación futura derivadas de los resultados obtenidos en esta tesis se resumieron en el **Capítulo 10**.

Abstract

Conventional technologies for wastewater treatment (WWT) such as activated sludge or anaerobic digestion entail high operating costs due to the high O_2 requirements for organic matter and/or NH_4^+ oxidation and to the necessity to increase nutrient removal from anaerobically digested WW, respectively. In this context, WWT by algal-bacterial systems constitutes a cost-efficient and environmentally friendly alternative to their biological counterparts. Algal-bacterial photobioreactors are based on the oxidation by heterotrophic microorganisms of the organic carbon present in the WW to CO_2, which is assimilated by microalgae during photosynthesis (in the presence of light) together with the inorganic carbon and nutrients present in the WW (mainly nitrogen and phosphorus). The O_2 produced during photosynthesis is in turn used by heterotrophic and autotrophic microorganisms to oxidize the organic carbon and NH_4^+ from WW, respectively, which constitutes an important energy saving compared to conventional mechanically aerated systems. Likewise, the biomass generated in the process can be used as a feedstock for biofuel production or as a biofertilizer. On the other hand, this biotechnology allows the integration of WWT and biogas upgrading or flue gas treatment (the CO_2 content in these gases can enhanced nutrient removal by assimilation into algal biomass). However, technical improvements and an optimization of the process must be conducted in order to ensure its economic viability and sustainability prior to the implementation of this technology in conventional wastewater treatment plants (WWTPs). In this context, particular attention should be given to technical limitations such as the high biomass harvesting cost or the high hydraulic retention time (HRT) required for WWT. Therefore, this thesis focused on the determination of the potential and the overcoming of the current limitations of algal-bacterial processes prior to a successful technology scale-up.

The state of the art about TAR by algal-bacterial system is presented in **Chapter 1**, while the objectives and development of the thesis are summarized in **Chapter 2**.

The high variability in the composition of WWs entails the need to determine their potential limitations for an optimum treatment by algal-bacterial processes. In this regard, the biodegradability by algal-bacterial systems of five agroindustrial wastewaters (potato processing WW (PW), fish processing WW (FW), animal feed production WW (MW), coffee manufacturing WW (CW) and yeast production WW (YW)) was investigated in enclosed batch biodegradation tests in **Chapter 3**. These WWs were selected based on their origin (representative agroindustries of Castilla-León) and on their high concentrations of carbon and nutrients. These WWs were also chosen based on their high variability in composition, which

allows elucidating the range of application and limitations of algal-bacterial systems during their WWT. The results showed that high concentrations of NH_3 (≈100 mg $N\text{-}NH_4^+$ L^{-1} at pH 8) and high pHs (≥10) inhibited the activity of the algal-bacterial consortium. In this regard, some WWs should be previously diluted or the pH should be controlled (e.g by CO_2 addition) for an effective WWT by algal-bacterial processes. The optimum C/N/P (carbon/nitrogen/phosphorus) ratio of the WW to be treated by this biotechnology was estimated to ≈ 100/18/2 (g/g/g), biodegradable carbon being the main limiting component to obtain a high nutrient removal.

The high operating cost of biomass harvesting in conventional photobioreactors (often suspended growth high rate algal ponds (HRAPs)) requires the development of more cost-effective harvesting techniques. Thus, the removal of C, N and P from centrate and primary domestic WW was studied in **Chapter 4** in two open bioreactors of 31 L and 0.5 m^2 of cultivation surface with biomass attached onto their surfaces (biofilm), which allowed to obtain a rapidly settling biomass with a low water content. One of the bioreactors was operated under a light:dark illumination regime of 16h:8h at a photosynthetically active radiation (PAR) of 88±16 μmol m^{-2} s^{-1}, while the other bioreactor was operated under dark conditions. Hence, this research allowed the comparison of the performance of an algal-bacterial biofilm bioreactor and a bacterial biofilm bioreactor. The performance of the bioreactors was only effective during primary domestic WWT as a result of the severe carbon limitation during the treatment of centrate (similarly to chapter 3). The results showed similar total carbon removal (organic and inorganic C removals ≥80%) in both systems, regardless of the HRT (3-10 d). Carbon was removed by stripping and assimilation into biomass in the algal-bacterial biofilm bioreactor, while the high nitrification activity in the bacterial biofilm bioreactor decreased the pH to 5.8. Stripping at this low pH was the main C removal mechanism. On the other hand, although complete NH_4^+ oxidation took place in both bioreactors, N removal was twice higher in the algal-bacterial biofilm system (mainly by assimilation into biomass and stripping). Likewise, P was only efficiently removed via assimilation into biomass in the algal-bacterial biofilm bioreactor. In this regard, the highest C, N and P removals (91±3%, 70±8% and 85±9%, respectively) were recorded at a HRT of 10 d in the algal-bacterial bioreactor. However, the high water evaporation (0.5-6.7 L m^{-2} d^{-1}) of this system contributed to the deterioration of the effluent quality. The harvesting of the biomass by surface scratching in the algal-bacterial biofilm bioreactor (in the bacterial biofilm bioreactor biomass growth was negligible) was effective and centrifugation was not required

to dry the biomass. Finally, the high removal of C and N by stripping resulted into a low biomass productivity (max. 3.1 g m^{-2} d^{-1}).

In **Chapter 5**, the performance of an enclosed (tubular) and an open algal-bacterial biofilm photobioreactors was compared in order to prevent the high C and N removal by striping typically encountered in open algal-bacterial biofilm photobioreactors (and the high water evaporation) to finally increase biomass productivity. Both photobioreactors, with a total volume of 31 L and 0.5 m^2 of illuminated surface, treated primary domestic WW at a HRT between 5 and 10 d under a light:dark illumination regime of 16h:8 h at a PAR of 74±3 µmol m^{-2} s^{-1}. The results showed a similar organic carbon removal in both systems regardless of the operational conditions (63-97%). However, inorganic carbon and nutrient removal was always higher in the open system (particularly in terms of P removal by assimilation into biomass). The excessive algal-bacterial biomass growth during the first experimental stages caused biomass clogging in the tubes due to their small diameter (1 cm), which resulted in a low WWT performance. Similarly to the results obtained in Chapter 4, the most effective WWT was recorded at a HRT of 10 d in the open photobioreactor, with C, N and P removals of 89±2%, 92±5% and 96±2%, respectively. The C, N and P removal mechanisms were also similar to those identified in the previous chapter. During this experimental work, the biomass composition remained constant regardless of the photobioreactor configuration and operational conditions (C: 43.1±2.3%, N: 7.6±0.7% and P: 0.9% in the enclosed system and C: 44.8±1.5%, N: 8.5±0.6% and P: 1.0±0.2% in the open system). On the other hand, the microalgae population dynamics were also studied in both photobioreactors with the main objective to determine the most effective microalgae species for WWT. The results showed a lower biodiversity in the enclosed system due to its lower risk contamination. *Phormidium*, *Scenedesmus* and *Chlorella* were identified in both systems, which confirmed their high tolerance to pollution and suitability for WWT.

The results obtained in chapters 4 and 5 highlighted the difficulty of biofilm photobioreactor scale-up. In this context, the application at full scale of suspended growth conventional photobioreactors (e.g HRAPs) has been more extensively investigated. However, there are still some limitations to be overcome before their final application in conventional WWTPs and industries. An outdoors (Valladolid, Spain) 180 L HRAP was operated from April to October in **Chapter 6** in order to treat fish farm wastewater (this WW was also combined with domestic WW based on its low nitrogen content). This research aimed at studying the influence of the environmental conditions of the region (characterized by warm and dry summers) on the quality of the final treated effluent. Thus, maximum Chemical Oxygen Demand (COD), total

kjeldahl nitrogen (TKN) and total phosphorus (TP) removal efficiencies of 77±9%, 83±10% and 94±6%, respectively, were recorded at 10 d of HRT. The C and N present in the WW were removed via assimilation into biomass (52±12% and 74±22%, respectively) and stripping, phosphorus being removed exclusively by assimilation into biomass (69±23%). The high fraction of C removed by stripping together with low C and nutrient loading rates resulted in low biomass productivities (max. 5 g m^{-2} d^{-1}). Biomass harvesting was conducted in an 8 L settler located after the HRAP with a removal efficiency of 82±18%, which supported a total suspended solid concentration in the effluent below the EU Directive for WW discharge into the environment (35 mg total suspended solids (TSS) L^{-1}). This harvesting technique constitutes an economic alternative to conventional physicochemical or mechanical processes such as centrifugation or filtration. The main limitation of the system evaluated was its high water evaporation rates, which achieved a maximum of 15 L m^{-2} d^{-1} by the end of the summer. However, and despite the need to develop further strategies to decrease the water evaporation in HRAPs, the scale-up of this system with lower turbulence (via correct engine sizing) would significantly reduce the water footprint of the process.

The effectiveness of algal-bacterial systems for WWT shown in previous chapters supported the integration of WWT and biogas upgrading (to produce a biomethane to be injected into natural gas grids or used as a vehicle fuel). In this context, the integration of WWT and biogas upgrading by algal-bacterial systems was evaluated in **Chapter 7** in a photobioreactor of 180 L interconnected via recirculation of the cultivation broth to an external absorption column of 2.5 L. The system operated under a light:dark illumination regime of 16h:8h at a PAR of 104±25 µmol m^{-2} s^{-1} using diluted centrate as a free nutrient source (**Chapter 7.1**). Centrate was diluted to avoid microalgae inhibition by the high NH_4^+ concentrations of centrate (446±101 mg N-NH_4^+ L^{-1}) and to induce nutrient starvation in order to promote microalgae lipid accumulation. The HRAP was operated at 7 d of HRT regardless of the dilution of the centrate (30 or 70 times). The synthetic biogas used in **Chapter 7.1** contained CO_2 at 30%. The results showed a maximum CO_2 removal efficiency of 99%, concomitant with nitrogen and phosphorus removal efficiencies of 100% and 82%, respectively, using 70 times diluted centrate. However, the concentration of O_2 in the upgraded biogas was ≈20% at these operational conditions, which would hinder the injection of the treated biogas into natural gas grids (maximum allowed concentration of 0.3% in Spain). On the other hand, a low lipid content (2.9-11.2%) was recorded in the algal-bacterial biomass, even under the extreme conditions imposed by nitrogen limitation, which revealed that this biomass was not adequate for biodiesel production. The efficiency of biomass harvesting in an 8 L settler interconnected to the HRAP

was 95%, which confirmed the high efficiency of natural biomass settling recorded in chapter 6. Finally, the study of the dynamics of microalgae population showed a high variability and diversity of microalgae over the experimental period, without the predominance of a microalga strain during HRAP operation. The same experimental system under the same irradiation regime was operated in **Chapter 7.2** for the treatment of digested vinasse and raw vinasse (both in diluted form). The composition of the synthetic biogas was CO_2 (29.5%), H_2S (0.5%) and CH_4 (70%). This study was devised to decrease the O_2 content in the upgraded biogas. Hence, the minimum O_2 concentration recorded in the upgraded biogas was 0.7±0.2% when raw vinasse was fed directly into the absorption column (without dilution) in order to promote the microbial consumption of the dissolved O_2 for organic matter oxidation (which minimized O_2 desorption to the upgraded biogas) at a HRT of 7 d. At these operational conditions, the CO_2 and H_2S removals accounted for 72±1% and 100±0%, respectively. However, the large amounts of N_2 desorbed from the cultivation broth to the upgraded biogas due to the open configuration of HRAPs resulted into a low CH_4 concentration (81±2%), which was significantly lower than the required for biomethane injection (≥95%). The C, N and P removal efficiencies of the system for diluted raw vinasse were 72±4%, 74±3% and 78±5%, respectively. On the other hand, a maximum biomass productivity of 16.9±0.7 g m^{-2} d^{-1} was achieved when raw diluted vinasse was fed into the system, while biomass harvesting by gravity sedimentation was effective (98.6±0.5%) regardless of the operational conditions. Finally, the monitoring of the dynamics of microalgae and bacteria population revealed a high microbial diversity regardless of the operational conditions.

The integration of WWT and flue gas treatment was carried out in **Chapter 8**, which theoretically would increase nutrient removal efficiency in the process due to the increase in microalgae production. This experimental research was carried out outdoors (Almería, Spain) in semi-industrial HRAPs of 700, 800 and 850 L from July to December. The influence of pH (7, 8 or 9) and CO_2 source for pH control (pure CO_2 or CO_2 from flue gas) on the efficiency of primary domestic WWT (COD, total nitrogen (TN), TP and *Escherichia coli*), on the composition of the algal-bacterial biomass and on biomass productivity was evaluated. The HRAPs were operated at a HRT from 2.7 to 6.7 d, which entailed a significant decrease compared to previous experimental works in HRAPs. Average COD, TN, TP and *Escherichia coli* removal efficiencies of 84±7%, 79±14%, 57±12% and 93±7%, respectively, were recorded during the six operational months. The influence of pH on the evaluated parameters in the tested range (from 7 to 9) was negligible. However, pH control by CO_2 addition from flue gas showed a slightly superior performance in WWT compared to the use of pure CO_2 (likely as a result of

the enhanced dissolved O_2 removal from the cultivation broth mediated by the larger gas flow rate injected). The evaluation of the photobioreactors performance with and without CO_2 addition showed that CO_2 addition from flue gas did not increase the nutrient removal efficiency or biomass productivity, likely due to the high extent of C removal by stripping. Biomass productivity ranged from 17±1 g m^{-2} d^{-1} in July to 4±0 g m^{-2} d^{-1} in December. The highest C, N and P biomass content (64.8%, 12.6% and 2.4%, respectively) was recorded when pH was controlled with flue gas. On the other hand, the protein content in the biomass remained constant (38.2±3.3%), while the lipid and carbohydrate contents ranged from 5.8% to 23.0% and from 38.0% a 61.2%, respectively. Finally, the high values of maximum quantum yield (Fv/Fm) revealed that the high light irradiances in summer (up to 900 W m^{-2}) induced a decrease in microalgae activity.

The integration of HRAPs in conventional WWTPs has been considered for secondary and tertiary treatment. Likewise, several scenarios to reuse the harvested biomass in order to improve the economic and energy balance of this biotechnology in current WWTPs have been proposed. However, the previous and subsequent unit operations required for integration of HRAPs within a full WWT scheme in order to achieve the concentrations in the effluent required by the EU Directive must be identified. Furthermore, biosolids management and their associated costs for the revalorization of the harvested biomass in HRAPs are often disregarded. These limitations were considered in **Chapter 9** during the evaluation of the full integration of HRAPs in conventional WWTPs under two different configurations for a population of 2000 person equivalent. In the first configuration, the raw WW was primary settled after pretreatment and the harvested biomass in the settler (after secondary WWT in the HRAP) was anaerobically digested. In the second configuration, the total fixed solids were removed before WW was introduced into the HRAP while the harvested algal-bacterial biomass in the settler was first dewatered in a belt press and then dried in a solar drying. The calculations showed that, despite the benefit derived from anaerobic digestion (10.7 € per inhabitant every year), the management of the digestate (including storage and seasonal land disposal) would imply prohibitive operating costs (32.5 € per inhabitant every year), which could eventually compromise the implementation of this biotechnology in small populations scenarios. On the contrary, the drying of the undigested solids would allow the storage of the biosolids for up to 1 year and their further land application when convenient. This alternative would reduce the transportation costs by a factor of 13.

Finally, the conclusions and future work based on the results here obtained were summarized in **Chapter 10**.

List of publications

The following publications are presented as a part of this PhD thesis. Seven of them were published in international journals indexed in ISI web of Knowledge (Papers I to VII). Paper VIII has been submitted for publication in Environmental Science and Technology.

Paper I. Posadas E., Bochon S., Coca M., García-González M. C., García-Encina P. A., Muñoz R. (2014). Microalgae-based agro-industrial wastewater treatment: a preliminary screening of biodegradability, J. Appl. Phycol. 26: 2335–2345.

Paper II. Posadas E., García-Encina P. A., Soltau A., Domínguez A., Díaz I., Muñoz R. (2013). Carbon and nutrient removal from centrates and domestic wastewater using algal-bacterial biofilm bioreactors, Bioresour. Technol. 139: 50-58.

Paper III. Posadas E., García-Encina P. A., Domínguez A., Díaz I., Becares E., Blanco S., Muñoz R. (2014). Enclosed tubular and open algal-bacterial biofilm photobioreactor for carbon and nutrient removal from domestic wastewaters, Ecol. Eng. 67: 156-164.

Paper IV. Posadas E., Muñoz A., García-González M.-C., Muñoz R., García-Encina P. A. (2015). A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters, J. Chem. Technol. Biotechnol. 90 (6): 1094-1101.

Paper V. Posadas E., Szpak D., Lombó F., Domínguez A., Díaz I., Blanco S., García-Encina P.A., Muñoz R. (2016). Feasibility study of biogas upgrading coupled with nutrient removal from anaerobic effluents using microalgae-based processes, J. Appl. Phycol. DOI: 10.1007/s10811-015-0758-3.

Paper VI. Posadas E., Serejo M. L., Blanco S., Pérez R., García Encina P.A., Muñoz R. (2015). Minimization of Biomethane Oxygen Concentration during Biogas Upgrading in Algal-Bacterial Photobioreactors, Algal Res. 12: 221-229.

Paper VII. Posadas E., Morales M. M., Gómez C., Acién F. G., Muñoz R. (2015). Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways, Chem. Eng. J. 265: 239-248.

Paper VIII. Posadas E., Plouviez M., Muñoz R., Guieysse B. (2016). Nutrient removal and solid management restrict the feasibility of algal biofuels generation via wastewater treatment. Submitted for publication to Environmental Science and Technology.

Contribution to the papers included in the thesis

Paper I. I was responsible for the design, start-up and operation of the experimental set-up, results evaluation and manuscript writing under the supervision of Dr. Raúl Muñoz and Dr. Pedro A. García Encina. The wastewaters from the different factories were collected with Dr. Maria Cruz García-González. Sonia Bochon participated in the project for four months.

Paper II. I designed and constructed the photobioreactors and I was responsible for the start-up and operation of the process, results evaluation and manuscript preparation under the supervision of Dr. Raúl Muñoz and Dr. Pedro A. García Encina. Anna Soltau collaborated in the project for one month.

Paper III. I designed the enclosed tubular photobioreactor and I constructed the open biofilm photobioreactor. I was responsible for the start-up and operation of the experimental set-up, results evaluation and manuscript writing under the supervision of Dr. Raúl Muñoz and Dr. Pedro A. García Encina. Dr. Saúl Blanco carried out the microalgae population characterization.

Paper IV. I was responsible for the design, start-up and operation of the experimental set-up, results evaluation and manuscript preparation under the supervision of Dr. Raúl Muñoz and Dr. Pedro A. García Encina. Dr. Maria Cruz García-González provided the fish farm wastewater to carry out the experimentation. Adriana Muñoz collaborated in the project for three months.

Paper V. I was responsible for the start-up and operation of the experimental set-up, results evaluation and manuscript writing under the supervision of Dr. Raúl Muñoz and Dr. Pedro A. García Encina. Dr. Saúl Blanco performed the microalgae population analyses and Dr. Felipe Lombó was in charge of the microalgae lipid content analyses. Dawid Szpak collaborated in the project for four months.

Paper VI. I was responsible for the design of the illumination system in collaboration with Mayara L. Serejo. Mayara L. Serejo was the main responsible for the operational work during the first operational stage. I carried out the operation of the experimental set-up, results evaluation and manuscript writing under the supervision of Dr. Raúl Muñoz and Dr. Pedro A. García Encina. Dr. Saúl Blanco conducted the microalgae population analyses and Dr. Rebeca Pérez carried out the DGGE analysis.

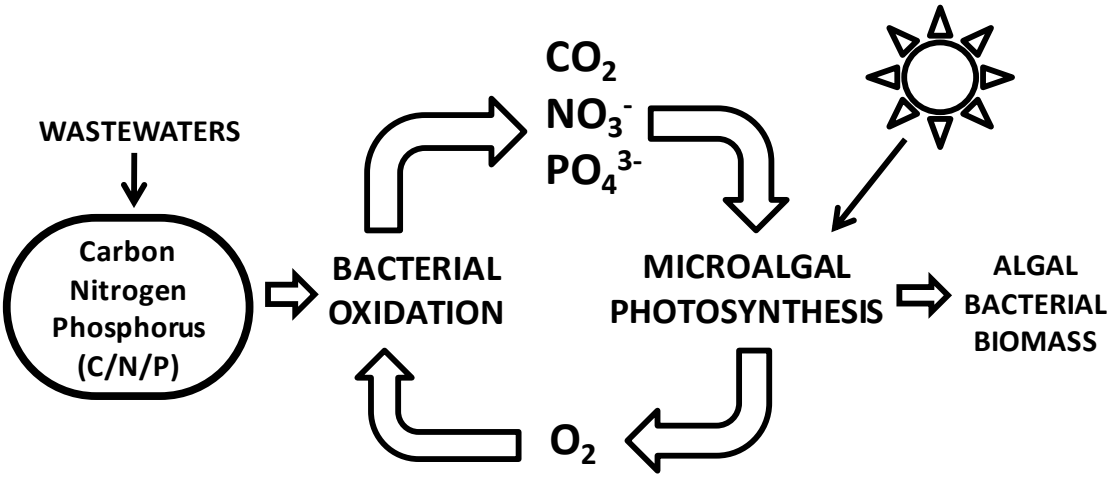
Paper VII. I carried the start-up and operation of the experimental set-up together with María del Mar Morales and Cintia Gómez. I wrote the manuscript in collaboration with María del Mar

Morales and under the supervision of Dr. F. Gabriel Acién and Dr. Raúl Muñoz. This research was carried at the facilities of Las Palmerillas (University of Almería, Spain).

Paper VIII. I conducted the initial literature review and analysis, along with the calculations of the simulation of the HRAP performance under the supervision of Dr. Benoit Guieysse. Maxence Plouviez provided the data to estimate N_2O emissions from the performance evaluation of a 1000 L HRAP located in Palmerston North (New Zealand). I collaborated with Maxence in the monitoring of this pilot HRAP for four months. I wrote the manuscript in collaboration with Dr. Benoit Guieysse and Dr. Raúl Muñoz. This research was carried at Massey University (Palmerston North, New Zealand).

Introduction

Chapter 1



1.1 Wastewater pollution

The world human population has increased from 2.6 to 7.2 billion people from 1950 to 2015. This growth has been mainly driven by the widespread implementation of modern medicine and improvements in working conditions, nutrition and food quality after World War II (Fig. 1) (Lee, 2003). The world human population is estimated to reach 9.4 billion people by 2050 and stabilized by 2110 when fertility rate decreases in developing countries (Pimentel and Burgess, 2015; United States Census Bureau, 2013).

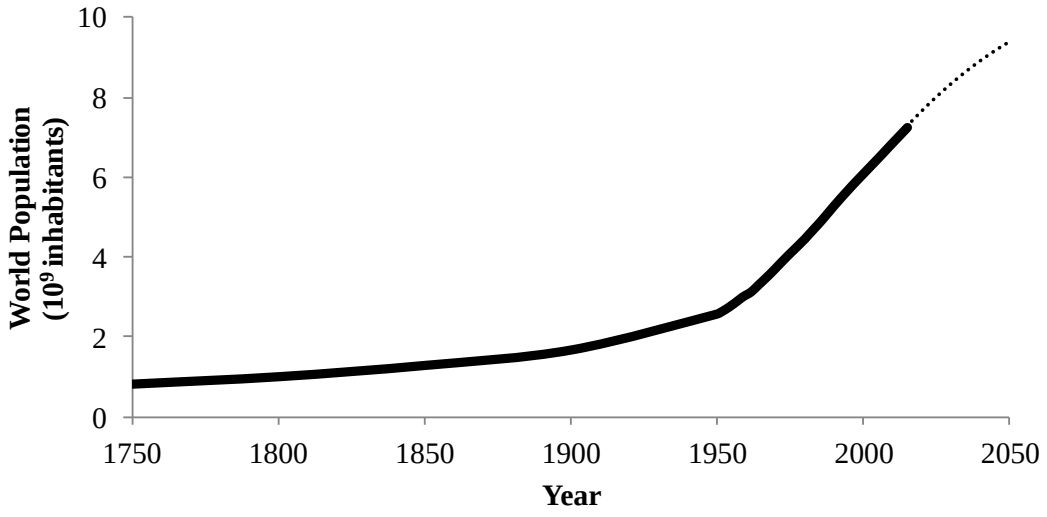


Figure 1. World human population increase (Source: United States Census Bureau, 2013): the bold line represents the real population, while the thin line refers to the estimated population.

Wastewater (WW) generation has also increased concomitantly to the increase in world human population (Fig. 2). Thus, WW generation worldwide (including domestic, commercial and industrial effluents in urban areas) averaged 275 billions of $m^3 year^{-1}$ in the period 2008-2012 (Aquastat, 2015). Despite their variable nature, WW are mainly characterized by their high concentrations of chemical oxygen demand (COD), which range from ≈ 400-500 mg L^{-1} in domestic WWs to 350000 mg L^{-1} in effluents from chemical industries (Metcalf and Eddy, 2003; Wesley, 2000). WWs also present high concentrations of total suspended solids (TSS), nitrogen, phosphorus and pathogens (the latter mainly found in domestic wastewaters) (Rooke, 2003). Likewise, heavy metals, cyanide, toxic organics or priority pollutants (e.g. benzene) are typically found in industrial wastewaters (Wesley, 2000). The uncontrolled disposal of these WWs into natural water bodies such as lakes or rivers causes a severe environmental damage derived from the depletion of dissolved oxygen concentration, eutrophication and contamination of surface and groundwater (Wesley, 2000).

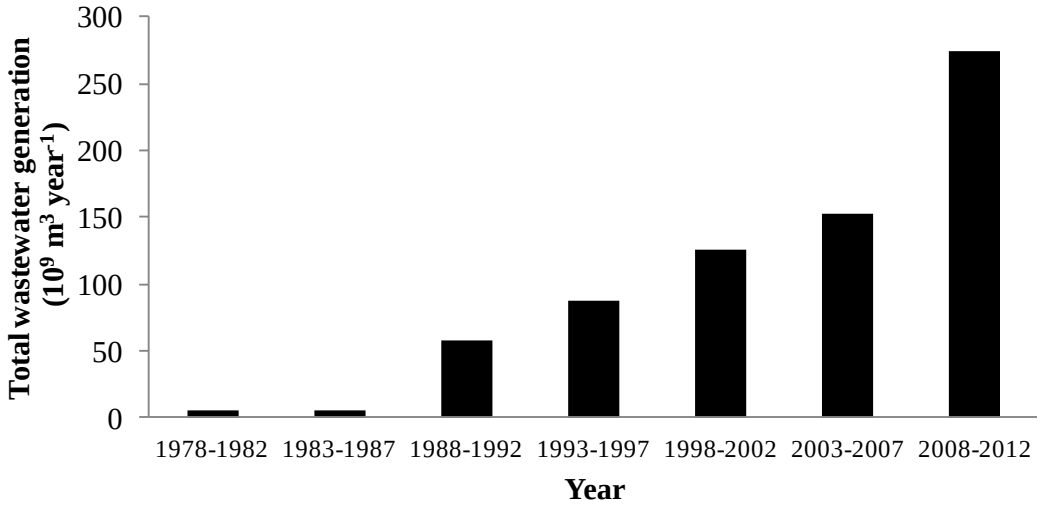


Figure 2. Total wastewater generation (Source: Aquastat, 2015).

Other parameters of WW that can affect the quality of the natural receiving water bodies are the pH and temperature of the effluent and its color and turbidity, which can hamper light penetration (Metcalf and Eddy, 2003). In addition, WW disposal promotes mal odors emissions and several human illnesses such as gastroenteritis, acute respiratory disease and eye, ear and skin infections (Dwight et al., 2005). For instance, the total number of people affected by diseases associated to a poor water management in the period 1998-2002 accounted for 655 and 866 per 1000 inhabitants in Burkina Faso and the Philippines, respectively (Aquastat, 2015). Therefore, a cost-efficient WW treatment is a current mandatory necessity worldwide.

1.2 Conventional technologies for wastewater treatment

The first WW management strategies were focused on domestic WW treatment because most municipal WW generated prior to the industrial development originated from domestic sources (Metcalf and Eddy, 2003; Rooke, 2003). The main steps, objectives and techniques traditionally used for WW treatment are shown in Table 1 (Wesley, 2000; Rooke, 2003; Metcalf and Eddy, 2003). A conventional wastewater treatment plant (WWTP) based on mechanical aeration (normally activated sludge processes) for organic matter and nutrients (mainly NH_4^+) oxidation requires a net annual energy input of ≈40 kWh per population equivalent (Meerburg et al., 2015). This demand of electricity corresponds to approximately 50%-60% of the total operating costs in conventional WWTPs, which compromises their economic and environmental sustainability (Meerburg et al., 2015; Curtis, 2010). In addition, activated sludge processes based on denitrification-nitrification or supplemented with

chemical precipitation contribute to a significant loss of nutrients. On the other hand, anaerobic processes have been also widely applied for WW and sludge treatment (Wesley, 2000; Rooke, 2003; Meerburg et al., 2015). Despite this technology allows for the production of renewable energy in the form of biogas (with a CH_4 content of ≈ 40-75%), its poor nutrient removal capacity and the low temperatures prevailing in most European and North American countries have limited its widespread application (Meerbug et al., 2015; Ryckebosch et al., 2011; Wilkie and Mulbry, 2002).

Table 1. Wastewater treatment steps, objectives and conventional technologies.

WASTEWATER TREATMENT STEP	OBJECTIVE	CONVENTIONAL TECHNOLOGIES
PRIMARY	Removal of the largest particles that can cause operational problems during WW treatment and removal of a fraction of the suspended solids and organic matter content in the raw WW	- Sedimentation - Screening - Flotation - Filtration - Chemical precipitation
SECONDARY	Removal of biodegradable organic matter (dissolved or suspension) and the remaining suspended solids	- Lagoons - Aerated lagoons - Activated sludge - Trickling filters - Anaerobic digesters
TERTIARY	Removal of nutrients (mainly nitrogen and phosphorus) and pathogen disinfection	- Nitrification/Denitrification - Biological Phosphate Removal - Chemical Precipitation - Chlorination - Ozonation -UV-treatment
QUATERNARY	Removal of toxic compounds and non-biodegradable suspended materials	- Activated Carbon - Chemical Flocculation
QUINARY	Removal of heavy metals	- Osmosis - Distillation - Electrodialysis

The above mentioned limitations of conventional WW treatment technologies, together with the higher recalcitrance of industrial wastewaters and the stricter wastewater discharge regulations (Table 2), have promoted the development of innovative wastewater treatment techniques (Wesley, 2000; Metcalf and Eddy, 2003; Curtis, 2000). For instance, the new designs of membrane bioreactors producing less sludge than conventional activated sludge processes have been successfully applied to the treatment of domestic and industrial WW, although membrane fouling and the need for hazardous cleaning chemicals still limit the

technical and environmental performance of this technology (Ahmad et al., 2013). Likewise, microbial fuel cells are anaerobic systems where the organic carbon content present in the WW is hydrolyzed, fermented and broken down into simpler compounds, whose electrons are donated to an electrode with the concomitant production of electricity (Curtis, 2010). However, microbial fuel cells have been only operated at a lab/pilot scale and often with synthetic wastewater at moderate temperatures (Curtis, 2010). Ion exchange-based WW treatment allows for a successful nutrient ($N\text{-}NH_4^+$, NO_3^-, PO_4^{3-}) and heavy metal removals at the expenses of prohibitive operating costs (Bochenek et al., 2011). Other chemical techniques such as the addition of chelating agents or metals (Al (III), Ca^{2+}, Fe (III) and Fe (II)) are effective for wastewater treatment but they involve a higher ecotoxicity compared to biological techniques (Ioannou et al., 2015). In this context, the development of cost-effective and environmentally friendly technologies is a must in order to achieve a sustainable wastewater treatment.

Table 2. Maximum COD, TN, TP and TSS concentrations allowed for WW discharge (Directive 2000/60/EC).

Parameter	Limit of discharge (mg L^{-1})
Chemical Oxygen Demand (COD)	125
Total nitrogen (TN)	15
Total phosphorus (TP)	2
Total suspended solids (TSS)	35

1.3 Algal-bacterial processes for the treatment of wastewater

Algal-bacterial processes have emerged as a promising low-cost, environmentally friendly and sustainable biotechnology for WW treatment able to solve the main drawbacks of the above described technologies (De Godos et al., 2009). The synergistic interactions between photosynthesis and heterotrophic/nitrifying metabolism support the oxidation of the organic matter and nitrogen (mainly as $N\text{-}NH_4^+$) present in the wastewater into CO_2 and $N\text{-}NO_3^-$, respectively, using the O_2 photosynthetically produced. These bioreaction products, together with the $P\text{-}PO_4^{3-}$ of the wastewater and in the presence of light, can be assimilated by microalgae and bacteria in photobioreactors (Muñoz and Guieysse, 2006) (Fig. 3). Likewise, microalgae-based processes allow for the removal of pathogens such as *Escherichia Coli* or *Vibrio cholera* as a result of solar (UV) light penetration and the increase in pH and dissolved oxygen concentrations mediated by algal photosynthesis (Heubeck et al., 2007; Craggs et al., 2004; Schumacher et al., 2003). Furthermore, algal-bacterial processes can remove heavy metals by biosorption or by precipitation as a consequence of the above mentioned pH

increase (Oswald, 2003; Muñoz and Guieysse, 2006). Finally, this technology has been also successfully applied for vet antibiotics and emerging organic contaminants removals from livestock effluents and urban wastewaters, respectively (De Godos et al., 2012; Matamoros et al., 2015).

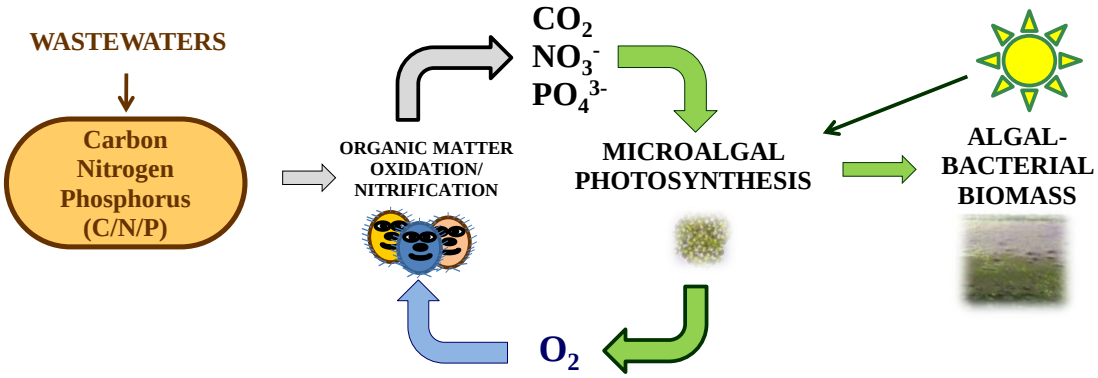


Figure 3. Algal-bacterial synergistic interactions during wastewater treatment: organic carbon and nutrient removal concomitant with algal biomass production.

The low-cost oxygen production by microalgae offers an economic advantage compared to the mechanical oxygenation of activated sludge processes (De Godos et al., 2009b). The higher nutrient assimilations as a result of the high biomass production rates in algal-bacterial photobioreactors represent another advantage over anaerobic and activated sludge processes (De Godos et al., 2009b; González et al., 2008). On the other hand, the high cost of reagents acquisition for phosphorus precipitation in conventional WWTPs and the high energy requirements for reverse osmosis applied for heavy metals removal might be overcome by algal-bacterial processes based on the photosynthetically induced pH increase and high biomass productivities (Wesley, 2000; Muñoz and Guieysse, 2006; Kumar et al., 2015). Likewise, algal-bacterial processes could replace the use of chlorine or ozone for pathogens removals (Giannakis et al., 2015; Verbyla and Mihelcic, 2015). For instance, Posadas et al. (2015) recorded average *E. Coli* removal efficiencies of 93±7% during secondary domestic wastewater treatment in three outdoors photobioreactors located in Almería (Spain) from July to December. In this context, secondary, tertiary, quaternary and quinary conventional WW treatment technologies showed in table 1 could be reduced eventually to one single step process by implementing microalgae-based photobioreactors (De la Noüe et al., 1992).

The first applications of algal-bacterial systems were carried out in the mid 50's for domestic WW treatment in California in the open photobioreactors namely high rate algal ponds (HRAPs) (Oswald, 1988). Since then, successive studies have extended the application of algal-

bacterial processes to industrial, agro-industrial and livestock effluents under different photobioreactor configurations (Muñoz and Guieysse, 2006; Muñoz et al., 2009; De Godos et al., 2009; Zamalloa, 2013).

1.3.1 Parameters influencing wastewater treatment in algal-bacterial processes

a. Environmental parameters

a. 1 pH

Based on the fact that the optimum pH for bacterial growth is slightly lower than that of microalgae (≈6.5-7.5 compared to ≈7.5-9.0), the optimum pH for WW treatment in photobioreactors ranges from 7 to 9 (Posadas et al., 2015; Metcalf and Eddy, 2003; Arbid et al., 2013). The pH of the cultivation broth depends on photosynthetic activity, algal/bacterial endogenous respiration, alkalinity and ionic composition of the target wastewater and the activity and type of autotrophic and heterotrophic metabolisms (Park et al., 2011b). Thus, pH increases during photosynthesis as a result of microalgae CO_2 uptake, and, in the absence of pH control, this parameter can reach values of up to 11, which could even inhibit both microalgae and bacteria activity (Posadas et al., 2014; Park and Craggs, 2010). The pH in photobioreactors is typically controlled by CO_2 addition (pure CO_2 or flue gas) at a set point of 8 (Park et al., 2011b; Arbid et al., 2013). On the other hand, nitrifying activity decreases the pH in photobioreactors as a result of H^+ release, which could eventually reduce wastewater treatment efficiency (Metcalf and Eddy, 2003). In this context, base addition might be required when treating wastewaters with a low alkalinity in photobioreactors operated at a high nitrification activity (Posadas et al., 2013). On the contrary, wastewaters with a high alkalinity (i.e. high inorganic carbon (IC) concentrations) can maintain a constant pH during WW treatment due to their high buffer capacity (Posadas et al., 2015b; De Godos et al., 2009b).

a.2 Dissolved oxygen

Dissolved oxygen (DO) concentrations in the cultivation broth must be higher than 2 mg O_2 L^{-1} in order to support high microbial rates of organic matter oxidation and nitrification (Metcalf and Eddy, 2003). On the other hand, microalgae activity could be inhibited at oxygen concentrations higher than 25 mg O_2 L^{-1} (≈300-400% higher than air saturation) (Molina et al., 2001). Despite lower DO concentrations are typically recorded in algal-bacterial photobioreactors treating WWs than in photosynthetic mass cultivation systems, oxygen

saturation can reach values of up to 300% during the peak radiation hours when treating domestic WW despite bacterial consumption (Posadas et al., 2015). In this context, oxygen removal from the cultivation broth might be mandatory in photobioreactors in order to avoid bacterial inhibition. O_2 stripping is often carried out by air sparging in enclosed columns (enclosed systems) or in sumps (open systems, mainly HRAPs) (Molina et al., 2001; Mendoza et al., 2013b).

a.3 Temperature

Temperature is a critical factor that affects microbial activity during WW treatment. For instance, bacterial activity can double when temperature increases by 10ºC, while at temperatures higher than 28ºC the oxidation of NH_4^+ is faster than NO_2^- oxidation (Metcalf and Eddy, 2003). On the other hand, the optimum temperature range for algal-bacterial growth has been set at 15-30ºC (Arbid et al., 2013; Hu, 2004), although some authors have suggested this optimum range for microalgae growth to 28-35ºC (Pulz, 2001).

a.4 Irradiation

The type and intensity of culture irradiation are critical factors governing microalgae photosynthetic activity and therefore, oxygen production, pH and nutrient fixation into biomass (Raeesossadati et al., 2014). In this context, the photosynthetic active radiation (PAR) corresponds to the radiation in the wavelength range (from 400 to 750 nm) supporting photosynthesis, which represents ≈48% of the total solar energy (η_{PAR}) (Park et al., 2011b; Yen et al., 2012). The O_2 production in the presence of light and the assimilation of inorganic carbon (CO_2) into algal biomass (CH_2O) is described by the photosynthesis "Z scheme" (Park et al., 2011b):

Photo-System I (PS I): $2\ H_2O \rightarrow O_2 + 4\ H^+$ (1)

Photo-System II (PS II): $CO_2 + 4\ H^+ \rightarrow CH_2O + H_2O$ (2)

The light energy absorbed by microalgae is first stored as $NADPH_2$ and ATP, which are then used to produce new biomass. The photosynthetic process is the result of two sets of interconnected *light* and *dark* reactions (Fig. 4) (Masojidek et al., 2004). Under optimum PS I and PS II operation, 8 electrons are necessary to carry out the complete photosynthesis with the subsequent production of 1 mol of CH_2O, which would correspond to a photosynthetic solar conversion efficiency of ≈33.8% ($\eta_{photosynthesis}$) (Park et al., 2011b).

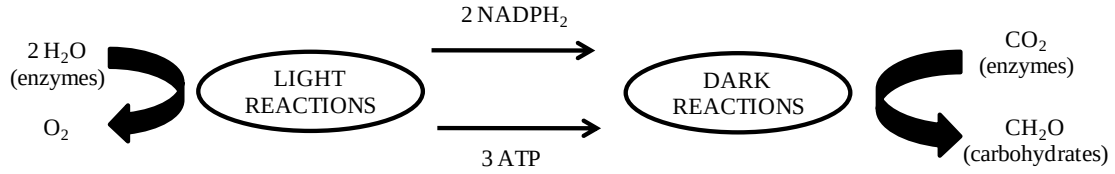


Figure 4. Basic scheme of the light and dark reactions underlying photosynthesis (Masodijek et al., 2004).

The influence of light intensity on microalgae growth is typically described by a P/I curve (Photosynthetic Rate/Light Intensity) accounting for three phases: i) light limitation: microalgae P increases proportionally to I and photosynthesis is limited by the rate of photons capture, ii) light saturation: constant microalgae P regardless of the increase in I, which corresponds to the maximum P and in this case photosynthesis is limited by the rate of the reactions following the capture of photons, and iii) light-inhibition: microalgae P declines at increasing I as a result of the deactivation of key proteins in the photosynthetic systems (Béchet et al., 2013). Light intensity must be homogeneously distributed along the entire microalgae culture in the photobioreactor at saturation light conditions in order to maximize biomass productivity, which is difficult to achieve at high biomass concentrations due to mutual shading (Yen et al., 2014). Photosynthesis in most microalgae species, at the above mentioned optimum temperature range, gets saturated at light irradiances of ≈200-250µmol m^{-2} s^{-1} (Torzillo et al., 2003; Ogbonna and Tanaka, 2000; Sousa et al., 2012; Talbot et al., 1991). This value corresponds to approximately 10% and 17% ($\eta_{saturation}$) of the summer and winter peak outdoors light irradiances (≈2500 µmol m^{-2} s^{-1} and ≈1200 µmol m^{-2} s^{-1}), respectively, in temperate latitudes (Park et al., 2011b; Burlew, 1953). Therefore, and based on the fact that ≈10-20% of the total solar radiation is lost by reflection ($\eta_{reflection}$) (at the above mentioned optimum values for microalgae cultivation) the maximum light irradiance that can be fixed by microalgae ranges from 1.3% to 7% (also depending on the photobioreactor design) (η_{real}) (Park and Craggs, 2011b). These values were estimated using equation 3:

$$\eta_{real} = [\eta_{PAR} \cdot \eta_{photosynthesis} \cdot (1-\eta_{reflection}) \cdot \eta_{saturation}] \cdot 100 \quad (3)$$

Based on these overall photosynthetic efficiencies (η_{real}), on the above mentioned light irradiances (G) in summer and winter expressed in MJ m^{-2} d^{-1}, and on the (E) specific heat value of the dry algal biomass (≈22.5 MJ Kg^{-1}), the maximum algal biomass productivity ($P_{productivity}$) that can be achieved in HRAPs accounts for ≈30 g m^{-2} d^{-1} (Park et al., 2011b). This productivity was calculated according to equation (4):

$$P_{productivity} = \frac{G \cdot \eta_{real}\,(\%)}{E \cdot 100} \quad (4)$$

Both the rate of photosynthesis and biomass productivity, when no other factors limit or inhibit microalgae growth, are determined by light availability in the cultivation broth (Molina et al., 1999). Hoffman (1998) reported algal biomass productivities in HRAPs and in periphyton photobioreactors to range between 10 and 35 g m^{-2} d^{-1}. Likewise, Posadas et al. (2015) recorded a maximum algal biomass productivity value of 17±1 g m^{-2} d^{-1} and a minimum of 4±0 g m^{-2} d^{-1} at average light irradiances of 1972±1230 µmol m^{-2} s^{-1} (summer) and 1264±662 µmol m^{-2} s^{-1} (winter), respectively, during secondary domestic WW treatment in pilot HRAPs located in Almería (Spain).

WW treatment under outdoors conditions is subjected to the daily and seasonal variations of irradiance. Thus, microalgae growth and the oxidation of organic matter and NH_4^+ may be limited by light during the dawn and dusk, while the culture may be photo saturated during midday due to solar irradiances exceeding 2000 µmol m^{-2} s^{-1} (Molina et al., 1999). The degree of light saturation is often quantified using the culture maximum quantum yield (Fv/Fm), which estimates the photochemical yield of PS II and indirectly the chlorophyll cell fluorescence (Masojidek et al., 2004). The ratio Fv/Fm strongly decreases when light irradiance is above the saturation limit (Torzillo et al., 2003). For example, Posadas et al. (2015) recorded a Fv/Fm increase from 0.33±0.03 to 0.59±0.01 in a 700 L HRAP treating secondary domestic wastewater when maximum light irradiance decreased from 3713 to 2394 µmol m^{-2} s^{-1}. Several operational and design strategies have been proposed in order to mitigate microalgae photoinhibition under outdoors conditions such as: i) increasing cell density and mixing, ii) tailored design of innovative photobioreactors to dilute the impinging irradiation or iii) searching for strains with small antenna size able to withstand to high oxygen concentrations. However, these approaches have resulted ineffective to mitigate photoinhibition in the long term (Torzillo et al., 2003).

On the other hand, inhibition of bacterial nitrifiying activity has been recorded by Vergara et al. (2016) at light irradiances higher than 250 µmol m^{-2} s^{-1}. This inhibition could have been caused by: i) a photoxidative damage of 400-430 nm photons to the enzyme ammonium oxygenase or its associated porphyrins co-factors, ii) an interference of the high light intensities during the synthesis of polypeptides involved in the ammonia monooxygenase system or iii) a damage in cytocromo-c (a protein involved in the electron transport chain). Nevertheless, this pernicious effect is alleviated at high microalgae culture densities, which rapidly decrease the average light irradiance within the culture.

a.5 Water evaporation rate

Water evaporation rates constitute another influential parameter that determines both the performance of the open photobioreactors (effluent concentrations) and the environmental sustainability of the process (water footprint). The evaporation rate depends on environmental parameters such as air and water temperature, solar irradiation, relative humidity and wind speed, photobioreactor turbulence and culture thickness (Murphy and Berberoglu, 2012). Guieysse et al. (2013) estimated the water footprint of outdoors 25 cm deep HRAPs operated at 7 d of hydraulic retention time (HRT) in Arid, Mediterranean, Subtropical, Temperate and Tropical regions to 6.2, 3.6, 3.2, 2.0 and 1.3 L m^{-2} d^{-1}, respectively. Likewise, Murphy and Berberoglu (2012) recorded evaporation losses of 6.0, 7.3, 3.4 and 1.0 L m^{-2} d^{-1} in the spring, summer, fall and winter, respectively, in algal biofilm photobioreactors at simulated environmental conditions similar to those found in Memphis (USA). Water evaporation prevents the increase in temperature to inhibitory values (Boelee et al., 2014). Nevertheless, water evaporation losses could eventually compromise the quality of the treated wastewater in open algal-bacterial photobioreactors due to the inherent increase in pollutant concentrations in the treated effluent (Posadas et al., 2015b; Matamoros et al., 2015). Some authors have proposed the use of cooling systems in order to decrease these high evaporative rates or the addition of fresh water to replace water losses (Murphy and Berberoglu, 2012; Alcántara et al., 2015b). However, these actions might compromise the environmental and economic sustainability of microalgae-based wastewater treatment.

b. Wastewaters characteristics

b.1 Carbon, nitrogen and phosphorus composition

The carbon (C), nitrogen (N) and phosphorus (P) content of the algal-bacterial biomass cultivated in wastewaters ranges from 40% to 60%, from 4% to 12% and from 0.5% to 2%, respectively (Posadas et al., 2014; 2015; Domínguez Cabanelas et al., 2013). The type of wastewater and the concentration of these macro nutrients can influence the final biomass composition (Serejo et al., 2015). Posadas et al. (2014) concluded that, in the absence of inhibitory or recalcitrant compounds, the initial C/N/P ratio of a wastewater was correlated with its biodegradability, the optimum biodegradability ratio being 100/18/2. In this context, most research studies evaluating wastewater treatment (domestic, industrial, agroindustrial and livestock effluents) using microalgae-based processes were operated under carbon limitation (Park and Craggs, 2010; Arbid et al., 2013). CO_2 addition from flue gas or biogas

could partially mitigate the above mentioned carbon deficiency (Park and Craggs, 2010; Bahr et al., 2014).

b.2 Inhibitory compounds

The presence of toxic inhibitory compounds in WW, even at moderate or low concentrations, can severely affect the activity of microalgae and bacteria and therefore the performance of WW treatment.

- **N-NH_4^+**, at concentrations higher than ≈100 mg N-NH_4^+ L^{-1} and pHs ≈7-8, can inhibit photosynthetic activity in some microalgae (or cyanobacteria) species (Posadas et al., 2014). This inhibitory effect increases at high pHs based on the aqueous NH_4^+ equilibrium (González et al., 2008; Alcántara et al., 2013):

  $$NH_4^+ + OH^- \rightarrow NH_3 + H_2O \ (pK_a=9.25) \quad (5)$$

  In this context, effluents with high NH_4^+ concentrations such as livestock wastewaters (≈600-3000 mg N-NH_4^+ L^{-1}), centrates (≈400-800 mg N-NH_4^+ L^{-1}) or anaerobically digested agroindustrial effluents (≈600-800 mg N-NH_4^+ L^{-1}) need to be previously diluted or provided at low loading rates in order to avoid microalgae inhibition (Posadas et al 2015c; De Godos et al., 2009; Serejo et al., 2015).

- **Heavy metals** can inhibit bacterial growth, photosynthesis and even generate morphological modifications in the microalgae cell walls at very low concentrations (Muñoz and Guieysse, 2006; Gopinath et al., 2011). The most common heavy metals found in WW include Cu, Cd, Cr, Hg, Pb and Zn (Kumar et al., 2015). Muñoz et al. (2006) observed *Chlorella sorokiniana* inhibition at Cu (II) concentrations of 2 mg L^{-1}, while Heng et al. (2004) reported that Cd (II) and Pb (II) inhibited the growth of *Anabeanaflos-aquae* by 50% at concentrations of 0.15 and 1 μg L^{-1}, respectively.

- **Toxic organic pollutants** such as salicylate, phenol or phenanthrene can also decrease the activity of microalgae and bacteria (Borde et al., 2003). Microalgae have been reported to be more easily inhibited in the presence of hazardous compounds than their bacterial counterparts. For instance, Borde et al. (2003) found a complete inhibition of *Chlorella sorokiniana* growth at 10 mg phenanthrene L^{-1}, while a *Pseudomonas* strain used in symbiosis with this microalga was able to biodegraded phenanthrene at 25 mg L^{-1}. More resistant microalgae and bacterial strains to high pollutants concentrations can be obtained by genetic manipulation, by cell adaptation to progressively higher pollutant concentrations or by isolation of strains from heavily

contaminated sites (Malik, 2004). For instance, Serejo et al. (2015) isolated a *Chlorella vulgaris* strain from a vinasse storage pond of a sugar and ethanol industry located in Mato Grosso do Sul (Brazil) and adapted this microalga strain to diluted anaerobically digested vinasse collected from a food industry located in Valladolid (Spain).

c. Operational conditions

c.1 Hydraulic retention time

The HRT measures the average length of time that the WW remains in the photobioreactor and directly determines the carbon and nutrient loads supplied to the photobioreactor and therefore the biomass productivity (Arbid et al., 2013; Metcalf and Eddy, 2003). The HRT (d) can be calculated using equation 6:

$$HRT = \frac{V}{Q} \quad (6)$$

where V is the photobioreactor volume (L) and Q the influent WW flow rate (L d^{-1}) The HRTs reported for WW treatment in photobioreactors can range from 23 d in a 180 L indoor HRAP treating seven times diluted centrate to 2.7 d for secondary domestic WW treatment under outdoors conditions in Almería (Spain) in 700-850 L HRAPs (Posadas et al., 2015; Bahr et al., 2014). These values are significantly higher than the typical HRTs applied in activated sludge or UASB reactors (≈3-12 h and ≈5-24 h, respectively) (Metcalf and Eddy, 2003). Therefore, the HRTs of algal-bacterial photobioreactors should be decreased in order to compete with the conventional technologies in terms of footprint and investment costs. An optimization of the HRT must be conducted for each photobioreactor configuration-wastewater-environmental conditions scenario in order to avoid the overload or under-use the natural purification capacity of algal-bacterial systems (Posadas et al., 2014b; Arbid et al., 2013). For instance, Posadas et al. (2013) recorded a phosphorus removal efficiency decrease from 57±17% to 36±22% when the HRT was decreased from 5.2 d to 3.1 d during secondary domestic wastewater in a 31 L algal-biofilm photobioreactor.

c.2 Mixing

Mixing is necessary to prevent the settling of microalgae and bacteria (which could cause anaerobic biomass decomposition); to avoid thermal and nutritional stratification inside the reactor; to remove the photosynthetically generated oxygen and to provide light access to microalgae, which would ultimately determine the light regime and, therefore, biomass

productivity in suspended cultures (Tredici, 2004). Optimum cultivation broth velocities for biomass recirculation in HRAPs (the most conventional design for wastewater treatment in algal-bacterial systems) range from 15 to 30 cm s^{-1} (Tredici, 2004). Despite mixing constitutes the higher cost of this biotechnology, the energy required for proper mixing to achieve a successful organic matter oxidation and nitrification would be reduced from 40 KWh person equivalent (P.E.)$^{-1}$ y^{-1} in activated sludge processes to ≈ 7 KWh $p.e^{-1}$ y^{-1} (Posadas et al., 2016b). Mixing also determines water evaporation losses and CO_2/NH_3 stripping since this parameter directly impacts the liquid-gas mass transfer coefficient (Posadas et al., 2013). Likewise, the mixing device is an important factor influencing the shear stress exerted on the microbial community, which is a key issue due to the fragility of most microalgae and bacterial cells (Barbosa et al., 2004). Mendoza et al. (2013) optimized the energy cost and liquid mixing in a 100 m length and 1 m wide channel HRAP at different depths (from 10 to 30 cm) concluding that mixing in a HRAP takes place mainly in the sump, paddlewheel and bends.

c.3 Other operational conditions

Other operational parameters such as the external CO_2 supply, that can influence both the performance of wastewater treatment in algal-bacterial processes and the extent of the carbon and nutrient removal mechanisms are below discussed.

1.3.2 Mechanisms of C, N and P removal in algal-bacterial processes

a. Carbon removal

Assimilation into biomass (biotic) and CO_2 stripping (abiotic) are the major mechanisms underlying carbon removal from wastewaters in open algal-bacterial photobioreactors (Posadas et al., 2014; 2015). Inorganic carbon is assimilated by microalgae during photosynthesis and by nitrifying bacteria according to the following biochemical processes (Metcalf and Eddy, 2003):

$$4.92\ CO_2 + 0.99\ H_2O + 0.15\ NO_3^- + 0.0094\ PO_4^{3-} \rightarrow CH_{1.7}O_{0.4}N_{0.15}P_{0.0094} + 1.52\ O_2 + 0.28\ H^+ \quad (7)$$

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7O_2N + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \quad (8)$$

However, the fraction of carbon assimilated by nitrifying bacteria is significantly lower than that by microalgae and it could be in fact considered negligible even under high nitrification activities in algal-bacterial photobioreactors (Posadas et al., 2014b). On the other hand, the share of carbon removed by stripping is influenced by the IC concentration in the cultivation

broth, the pH and the liquid-gas mass transfer coefficients according to the CO_2 equilibrium (Metcalf and Eddy, 2003):

$$CO_2(l) + H_2O(l) \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

$$[pK_{a1}(H_2CO^3 \leftrightarrow HCO_3^-) = 6.35;\ pK_{a2}(HCO_3^- \leftrightarrow CO_3^{2-}) = 10.33] \quad (9)$$

The concentration of IC in the cultivation medium is determined by the IC concentration of the inlet wastewater, the external CO_2 supply (if any,) the total organic carbon (TOC) oxidized by bacteria and the IC consumption by microalgae and nitrifying bacteria (Posadas et al., 2013; 2015c). Therefore, at aqueous CO_2 concentrations higher than 0.38 mg L^{-1}, which corresponds to the CO_2 concentration in equilibrium with the atmospheric CO_2 concentration, IC is removed by stripping as a result of the positive concentration gradient from the aqueous phase to the atmosphere. Carbon removal by this abiotic mechanism, together with the low C/N ratio of most wastewaters, decreases the effectiveness of nutrient removal and biomass productivity (Posadas et al., 2013). Abiotic carbon removal can contribute to more than 50% of the total C removed from the wastewaters in open photobioreactors under low photosynthetic activities (Posadas et al., 2013; 2015; 2016). For instance, Posadas et al. (2014b) recorded a recovery (as harvested biomass) of only 13±4% of the total carbon removed during secondary domestic wastewater treatment in an indoor 31 L algal-bacterial biofilm photobioreactor when the pH decreased to 6.7±0.2 due to the intense nitrification activity.

b. Nitrogen removal

The nitrogen present in wastewaters can be removed by assimilation into biomass (biotic) and by NH_3 stripping (abiotic) in open algal-bacterial photobioreactors (Cai et al., 2013). Despite nitrogen assimilation into biomass can be carried out by microalgae and bacteria, an study conducted by Posadas et al. (2013) found twice higher nutrient (N and P) removals in algal-bacterial systems than in bacterial systems as a result of the additional photosynthetic IC fixation by microalgae. In fact, the high HRTs required in the algal-bacterial processes to carry out a successful wastewater treatment are due to the need to remove nitrogen (and phosphorous) by assimilation (Alcántara et al., 2015c). Microalgae can assimilate NO_3^-, NO_2^- or NH_4^+ through the biological reactions shown in figure 5, where all forms of inorganic nitrogen are ultimately reduced to ammonium prior to being incorporated into aminoacids. Thus, NH_4^+ constitutes the preferred inorganic nitrogen source by microalgae and bacteria because its assimilation involves the lower energy consumption. In this context, nitrite or nitrate consumption by microalgae does not occur until ammonium is almost completely depleted in

the cultivation medium (Cai et al., 2013). This facilitates microalgae cultivation in WW due to the fact that most of the total nitrogen (TN) content in WW remains in the form of NH_4^+ (Metcalf and Eddy, 2003). However, under non IC limiting conditions, the faster NH_4^+ oxidation (at DO ≥2 mg O_2 L^{-1}) compared to N assimilation into biomass or NH_3 stripping often results in the bioconversion of most of the TN content into NO_3^-, which involves high energy requirements for assimilation and a decrease in the TN removal efficiencies (Posadas et al., 2013; 2015; 2015b; Cai et al., 2013). For instance, Posadas et al. (2015) recorded a TN removal efficiency of 75±3% when nitrification accounted for 21.6±1.0% of the TN input in a 800 L HRAP during secondary domestic wastewater at pH 8, while this removal increased to 93±2% when nitrification activity was suppressed as a consequence of the low outdoors temperatures (and the negligible NH_4^+ oxidation rate promoted higher N removals by stripping).

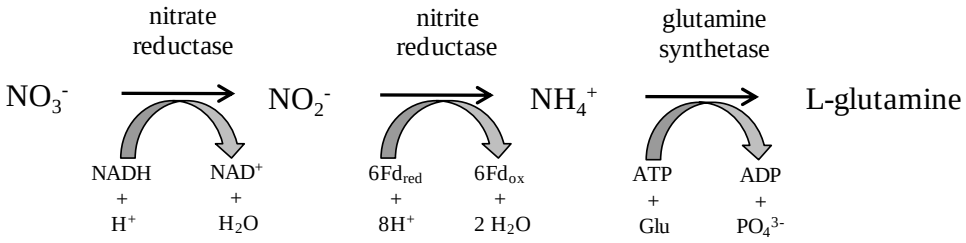


Figure 5. Assimilation of inorganic nitrogen by microalgae (Cai et al., 2013).

Abiotic nitrogen removal takes place by NH_3 volatilization, which depends on the pH and concentration of NH_4^+ in the cultivation broth and on the liquid-gas mass transfer coefficient (Metcalf and Eddy, 2003). Based on its negligible atmospheric concentration, the NH_3 concentration gradient from the cultivation broth to the atmosphere is always positive. In this context, Posadas et al. (2015) confirmed that NH_3 stripping from WW has an important contribution to TN removal in the absence of nitrification in algal-bacterial systems with low biomass concentrations (García et al., 2000b).

c. Phosphorus removal

Phosphorus (typically present in wastewaters as PO_4^{3-}) can be removed by assimilation into biomass (biotic) or by precipitation at high pHs (>9) (Cai et al., 2013; Heubeck et al., 2007). However, assimilation into biomass often represents the only P removal mechanism during WW treatment in algal-bacterial processes based on the optimum pH operational range (7-9) (Posadas et al., 2015; 2015b). Despite P biomass composition ranges from 0.5 to 2%, Domínguez Cabanelas et al. (2011) recorded a P content of 4% in microalgae grown in centrate. This large P content was likely mediated by a luxury phosphorus uptake mechanism

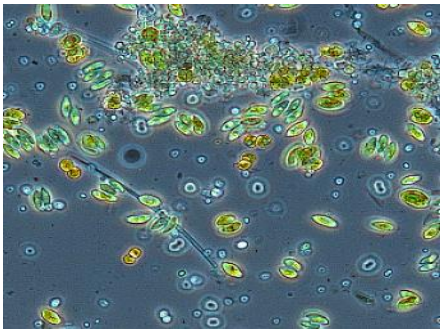
present in some microalgae species, which can be influenced by the phosphate concentration in the wastewaters, the light intensity and the temperature (Powell et al., 2008; 2009; Alcántara et al., 2013; 2015). Thus, phosphorus can be accumulated as polyphosphate by microalgae and then used as a P reserve when the external phosphorus concentration limits biomass growth or as an energy source (Brown and Shilton, 2014). Luxury uptake is competitive with intracellular phosphorus assimilation to sustain microbial growth and the optimization of the HRTs in algal ponds to boost polyphosphate accumulation during wastewater treatment is currently under studied (Brown and Shilton, 2014).

1.3.3 Microalgae and bacteria diversity

The top 6 most pollution-tolerant genera of microalgae reported are *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella* and *Nitzschia*, while the top 4 most pollution-tolerant reported microalgae species correspond to *Euglena viridis, Nitzschia palea, Oscillatoria limosa* and *Scenedesmus quadricauda* (Palmer, 1969). In this context, different species of Chlorophytes such as *Chlorella* and *Scenedesmus* have been tested for their effectiveness for carbon and nutrient removals from different types of wastewater (Cai et al., 2013). For instance, Arbid et al. (2014) evaluated the potential of *Scenedesmus obliquus*, *Chlorella vulgaris*, *Chlorella kessleri* and a natural Bloom of microalgae to support the treatment of urban and synthetic WW using air supplemented with 5% of CO_2.

High microalgae diversity is often found during the continuous treatment of different types of wastewaters under outdoors or laboratory conditions, with a higher microalgae diversity in open systems than in enclosed as a result of the higher contamination risk (Posadas et al., 2014b; 2015c; García et al., 2000). In this context, the predominance of the inoculated microalgae in the photobioreactors cannot be guaranteed during long term operation due to microalgae adaptability to the environmental and operational conditions, unless extreme cultivation conditions are employed as a selective pressure (De Godos et al., 2009). Thus, Serejo et al. (2015) inoculated with *Chlorella* sp. an indoor 180 L HRAP treating diluted anaerobically digested vinasse at 7 d of HRT and after 30 d of operation this species was replaced by *Pseudanabaena* sp. Monoalgal cultures are not common during the treatment of wastewaters in photobioreactors, despite a microalgae strain can eventually be predominant during a certain period of time (Photograph 1) (Posadas et al., 2014b; Serejo et al., 2015). The predominance of a target microalga species can be achieved by recirculation of the harvested biomass as recently shown by Park et al. (2011) or at high microalgae density cultures (≈2 g TSS

L^{-1}), as are the cases of the pilot HRAPs located in Almería and Chiclana de la Frontera (Spain) with cultivations of *Scenedesmus* and *Coelastrum* sp., respectively (Posadas et al., 2015; All-gas, 2013). Finally, higher microalgae diversity is typically found in outdoors cultures during summer as a result of the higher temperatures and light irradiances favoring microalgae growth (De Godos et al., 2009).

a)  **b)** 

Photograph 1. a) Microscopic view of a microalgae consortium during secondary domestic wastewater treatment (Predominance of *Scenedesmus*) (Posadas et al., 2014b); **b)** Microscopic view of filamentous microalgae and bacteria flocs during centrate treatment (Posadas et al., 2016).

On the other hand, Denaturing Gradient Gel Eletrophoresis (DGGE) analyses have been consistently carried out in algal-bacterial photobioreactors in order to determine the richness and composition of the bacterial community supporting WW treatment. Thus, Posadas et al. (2015c) recorded a high bacterial diversity (2.8-3.3) during the treatment of diluted anaerobically digested vinasse in a 180 L HRAP with simultaneous biogas upgrading based on the Shannon-Wiener diversity index (which indicates low and high bacterial diversity for 1.5 and 3.5, respectively) (McDonald, 2003). Similarly, Alcántara et al. (2015) found a high microbial diversity (Shannon-Wiener indices of 2.6-3.5) during the evaluation of WW treatment in a novel anoxic-aerobic algal-bacterial photobioreactor. On the contrary, Erkelens et al. (2014) recorded a Shannon-Wiener ranging from 0.5 to 3 during *Tetraselmis*-based treatment of digestate effluent. Different phyla of bacteria such as *Proteobacteria*, *Firmicutes* and *Chlamydiae* have been identified during the treatment of piggery and anaerobically digested vinasse effluents in HRAPs (Posadas et al., 2015c; Ferrero et al., 2012). Despite the understanding provided by these preliminary results, there is still a lack of systematic studies devoted to determine possible correlation between clusters of microalgae and bacteria during WW treatment, which will help elucidating on the underpinning mechanisms of symbiotic interaction between both groups. In this context, the application of advanced molecular

techniques will enable a more detailed characterization of the structure and functionality of the microbial populations and their mutualistic interactions.

1.4 Photobioreactors design

The optimization of photobioreactor design for wastewater treatment should be conducted based on the prevailing environmental conditions and the target extent of the C, N and P removal mechanisms. Thus, key factors to be considered during the construction of a lab, pilot or industrial scale photobioreactor are the optimization of light supply (high surface/volume ratio); the mixing and degassing efficiency (O_2/CO_2 exchange); maximum allowed water footprint and the carbon and nutrients supply at the minimum construction and operation costs (Posten et al., 2009; Tredici et al., 2004). Photobioreactors can be classified according to the type of biomass growth into suspended or biofilm systems.

a. Suspended growth photobioreactors

Suspended cell growth is the most common cultivation method for microalgae mass production (Katarzyna et al., 2015). HRAPs and tubular photobioreactors (TPBRs) constitute the most common open and enclosed photobioreactor configurations, respectively (Fig. 6) (Alcántara et al., 2015c).

HRAPs have been the most applied algal-bacterial photobioreactor configuration for the treatment of domestic (secondary or tertiary), livestock, agroindustrial and industrial WW (Table 3) (De Godos et al., 2009; Serejo et al. 2015; Razzak et al., 2013). HRAPs, also called raceways, are open shallow ponds with a paddle wheel to provide culture mixing and recirculation (15-30 cm s^{-1}) and the access of microalgae to light and nutrients (De Godos et al., 2009). The depth of the HRAPs ranges from 10 to 30 cm and some designs are provided with a sump and bends to improve mixing, CO_2 supply and O_2 removal (Mendoza et al., 2013; 2013b). The main advantages of this photobioreactor configuration are their relatively easy construction and operation, and their low operational and maintenance costs, while their main disadvantage is the low light photosynthetic efficiency (≈2.5%), which results in low biomass productivities (≈ 5 - 20 g m^{-2} d^{-1}) (Park et al., 2011; Saeid and Chojnacka, 2015). Typical biomass concentrations in HRAPs range from 0.3 to 1 g TSS L^{-1} with optimum HRTs between 3 and 10 d (Arbid et al., 2013; Posadas et al., 2015).

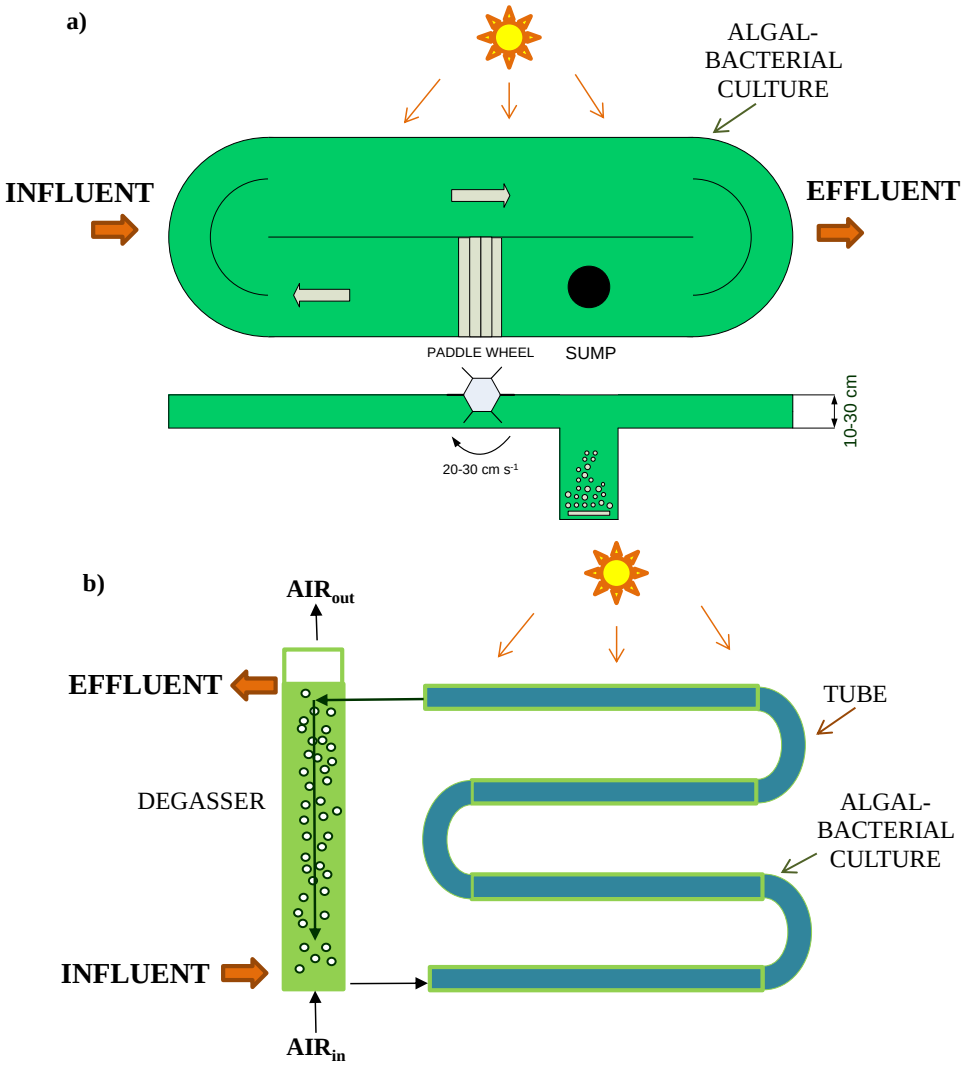


Figure 6. Photobioreactor configurations for suspended growth microalgae cultivation: **a)** High rate algal pond (open system); **b)** Tubular photobioreactor (TPBR) (enclosed system).

Table 3. Main results obtained during the treatment of wastewaters in HRAPs; O=outdoors; I=laboratory conditions (indoor); RE= removal efficiency.

Wastewater	Gas treatment	Illumination/City	Volume (L)	HRT (d)	Relevant information	Reference
Domestic	---	O: Barcelona (Spain)	570	HRAP A: 4-10 HRAP B: 3-8	Annual average TN-RE ≈ 73% (HRAP A) and ≈ 57% (HRAP B). NH_4^+ removal by stripping ≈ 47% (HRAP A) and ≈ 32% (HRAP B)	García et al., 2000b
Domestic	---	O: Barcelona (Spain)	500	HRAP A: 4 HRAP B: 8	Annual average COD-RE ≈ 66-85%; NH_4^+-RE summer ≈ 99%; Emerging contaminant removal ≥ 90%: caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate, oxybenzone	Matamoros et al., 2015
Swine manure	---	O: Valladolid (Spain)	464	10	COD-RE ≈ 76±11%; TKN-RE ≈ 88±6%; P-RE: ≈ 10%;Biomass productivity ≈ 21-28 g m^{-2} d^{-1}; Higher microalgae biodiversity in summer than in winter	De Godos et al., 2009
Fish farm + primary domestic	---	O: Valladolid (Spain)	180	7-20	Maximum values: COD-RE ≈ 77±9%; TKN-RE ≈ 83±10%; P-RE ≈ 94±6%; Biomass productivity ≈ 5 g m^{-2} d^{-1}; Evaporation losses ≈15 L m^{-2} d^{-1}	Posadas et al., 2015b
Synthetic sewage water	---	I: PAR ≈ 280 µmol m^{-2} s^{-1}/ light:dark cycles 12:12 h	7	7	CODs-RE ≈ 89%; TN-RE ≈ 80%; TP-RE ≈ 84%; Production of 0.005% g $N\text{-}N_2O$-g N input	Alcántara et al., 2015b
Slaughterhouse	---	O (HRAP A): Valladolid (Spain); I (HRAP B): I: 4500 ± 150 lux located 20 cm over the surface cycles 12:12 h	75	10-15	CODs-RE: ≈ 90%; TP-RE: ≈70-90%; Max. Biomass productivity ≈ 12.7 g m^{-2} d^{-1}; pH range ≈ 7-8.5; Biomass composition: lipids (12.8-14.8%), carbohydrates (12.6%-25.6%), proteins (45.1%-57.8%), ash (4.1%-5.5%)	Hernández et al., 2016

Wastewater	Gas treatment	Illumination/City	Volume (L)	HRT (d)	Relevant information	Reference
Secondary domestic	---	O: Cádiz (Spain)	530	10	Maximum values: TN-RE ≈ 60±1%; Biomass productivity ≈ 8.3±1.4 g m^{-2} d^{-1}; Lipid content (20.8±0.2%)	Arbid et al., 2013b
Diluted Centrates	Flue gas	O: Almería (Spain)	800	5	Production of microalgae at dilution rates only lower than 30%; Biomass concentration of ≈0.48 g TSS L^{-1} at 20%; Max. TN-RE ≈ 40%	Ledda et al., 2015
Primary domestic	Flue gas	O: Almería (Spain)	700; 800; 850	2.7±0.1-6.7±0.4	Average values: COD-RE ≈ 84±7%; TN-RE ≈ 79±14%; TP-RE ≈57±12%; *E. Coli*-RE ≈ 93±7%; Biomass productivity ≈ 4±0 to 17±1 g m^{-2} d^{-1}; Biomass composition: C (64.8%); N (12.6%); P (2.4%); Proteins (38.2±3.3%); lipids (6% to 23%); carbohydrates (38% to 61%)	Posadas et al., 2015
Secondary domestic	Flue gas	O: Cádiz (Spain)	533 (HRAP); 593(HRAP+S (sump)	8;8	Maximum values (HRAP+S) TN-RE ≈ 92.15±1.45%; TP-RE ≈ 95.10±0.84%; Biomass productivity ≈ 19.77±0.38 g m^{-2} d^{-1}; Biomass composition: C (40.0±1.0% to 47.5±2.05%); N (3.2±0.1% to 4.2±0.2%); Lipids (19.7±0.7% to 25.7±0.9%)	Arbid et al., 2013
Swine manure	Flue gas	O: Valladolid (Spain)	465	10	COD-RE ≈ 56±31%; NH_4^+-RE ≈ 98±1%; TP-RE ≤15%; Max. biomass concentration: HRAP A (CO_2 flue gas) ≈ 500 mg VSS L^{-1}; HRAP B (no CO_2 flue gas) ≈ 400 mg VSS L^{-1}	De Godos et al., 2010

Wastewater	Gas treatment	Illumination/City	Volume (L)	HRT (d)	Relevant information	Reference
Diluted centrates	Synthetic biogas	I: PAR ≈ 80 µmol m^{-2} s^{-1}/ light:dark cycles 24:0 h	180	23	CO_2-RE: ≈ 40±6%; H_2S-RE ≈100%; Biomass concentration ≈ 0.6±0.2 g TSS L^{-1}; pH:7	Bahr et al., 2014
Diluted anaerobically digested vinasse	Synthetic biogas	I: PAR ≈ 104±25 µmol m^{-2} s^{-1}/ light:dark cycles 16:8 h	180	7.4±0.3	CO_2-RE ≈ 80%; H_2S-RE ≈ 100%; Max. biomass productivity ≈ 12±1 g m^{-2} d^{-1}; Biomass composition: C (49±2%); N (9±0%); P (1±0%); High carbohydrate content ≈ 60%-76%	Serejo et al., 2015
Diluted centrates	Synthetic biogas	I: PAR ≈ 75±5 µmol m^{-2} s^{-1}/ light:dark cycles 24:0 h	180	7	CO_2-RE ≈ 99%; TN-RE ≈ 100%; TP-RE ≈ 82%; Low lipid content (2.9-11.2%); Settler Biomass RE: 95%	Posadas et al., 2016
Diluted anaerobically digested vinasse / Raw vinasse	Synthetic biogas	I: PAR ≈ 104±25mol m^{-2} s^{-1}/ light:dark cycles 16:8 h	180	7.4±0.2	CO_2-RE ≈ 72±1%; H_2S-RE ≈ 100%; Minimum O_2 level in the upgraded biogas of 0.7±0.2%; TC-RE ≈ 72±4%; TN-RE ≈ 74±3%; TP-RE ≈ 78±5%; Max. biomass productivity ≈ 16.9±0.7 g m^{-2} d^{-1}; Settler Biomass RE ≈ 98.6±0.5%	Posadas et al., 2015c
Diluted centrates	Pure CO_2	O: Hamilton (New Zealand)	8000	4-8	Recycling of harvested biomass increased algal harvesting RE from 60 to 85% and microalgae size ≈ 13-30%	Park et al., 2011
Diluted centrates	Pure CO_2	O: Hamilton (New Zealand)	8000	4-8	Max. biomass productivity ≈ 24.7 g m^{-2} d^{-1}; Biomass settling RE (8 d HRT) ≈ 83%; (4 d HRT) ≈ 69%	Park and Craggs, 2010

Wastewater	Gas treatment	Illumination/City	Volume (L)	HRT (d)	Relevant information	Reference
Primary domestic	Pure CO_2	O: Hamilton (New Zealand)	2. 23 m^2 of cultivation surface/ Different depth: 20/30/40 cm	4-9	$NH4^+$-RE: from 59±18% (40 cm depth) to 79±3% (20 cm depth); P_s-RE: from 12±10% (40 cm depth) to 34±26% (20 cm depth); pH: 9.2±0.2 (20 cm depth); 8.8±0.0 (30 cm depth); 8.6±0.0 (40 cm depth)	Sutherland et al., 2014b
Primary domestic	Pure CO_2	O: Christchurch (New Zealand)	4375000	5.5-9	Max. N-NH_4^+-RE ≈ 79±13%; Ps-RE ≈ 49±22%; pH range ≈ 9.0±0.4 to 9.7±.6; Maximum electron transfer rate (ERT_{max}) ≈ 1.73±0.26 µmol e^- mg *Chl-a*$^{-1}$ s^{-1}	Sutherland et al., 2014
Primary domestic	Pure CO_2	O: Christchurch (New Zealand)	4375000	8-9	BOD_5-RE ≈ 50%; N-NH_4^+-RE ≈ 65%; P_s-RE ≈19%; *E. coli*-RE ≈ 2 log; Biomass productivity: ≈ 8 g VSS m^{-2} d^{-1}; pH ≈ 9.1-9.3	Cragss et al., 2012

TPBRs have been traditionally applied to the mass cultivation of microalgae with industrial interest using synthetic mineral salt media and external CO_2 supply rather than for WW treatment mainly due to their lower contamination risk and the more efficient control of cultivation conditions compared to their open counterparts (Grobbelaar, 2010). The optimum diameter of the tubes in TPBRs ranges from 1 to 12 cm, since smaller diameters could cause biomass clogging (Posadas et al., 2014b; Posten et al., 2009; Acién et al., 2012). Likewise, the recommended linear velocity in the tubes should be between 20 and 50 cm s^{-1}, which is commonly controlled by the use of airlift systems or centrifugal pumps (Posten et al., 2009). Their higher ratio of illuminated surface to photobioreactor volume compared to HRAPs entails higher photosynthetic efficiency (up to 7%) and, consequently, higher biomass concentrations and productivities (up to 6 g TSS L^{-1} and up to 40 g m^{-2} d^{-1}, respectively) (Posten, 2009). The most typical HRT to support the above mentioned biomass concentrations and productivities in these TPBRs is 3 d (Acién et al., 2012). However, the main disadvantages of TPBRs are their high construction and operational cost (48 kWh m^{-3} WW treated), operational issues such as temperature control or oxygen removal and biofouling (Acién et al., 2012; Razzak et al., 2013). In this context, Arbid et al. (2013) reported deterioration in COD removal efficiency in a 350 L TPBR during tertiary wastewater treatment due to their high temperatures and low irradiances as a consequence of biofouling and hydrolysis of the attached biomass into the tubes (Table 4).

The low biomass concentrations typically encountered in suspended growth photobioreactors (<1% of dry algal biomass) entails the need to harvest large volumes of water to separate the treated WW from the suspended biomass produced (Katarzyna et al. 2015). Furthermore, the small size of microalgae cells, together with their strong negative surface charge and their density similar to water, increase the difficulty to separate the suspended algal-bacterial biomass after WW treatment (Park et al., 2011; Christenson and Sims, 2011). In this regard, microalgae harvesting often constitutes the bottleneck of the economic sustainability of microalgae-based WW treatment (Acién et al., 2012; Norsker et al., 2011). Despite the existence of several algal biomass harvesting techniques (Table 5), harvesting by gravity sedimentation is one of the most inexpensive and preferred techniques due to its simplicity (Barros et al., 2015). Posadas et al. (2015c) recorded an average harvesting efficiency of 98.6±0.5% by natural biomass settling during the treatment of diluted anaerobically digested vinasse and raw vinasse in a 180 L HRAP interconnected to a settler with a HRT of 23.5±0.3 min. Likewise, Park et al. (2011) enhanced the natural settleability of the algal biomass via recirculation of the readily settleable algal species in an 8000 L HRAP during secondary domestic wastewater treatment.

Table 4. Main results obtained during the treatment of wastewaters in TBPRs; O=outdoors; I=laboratory conditions (indoor); RE= removal efficiency.

Wastewater	Gas treatment	Illumination/City	Volume (L)	HRT (d)	Relevant information	Reference
Secondary domestic	---	O: Cádiz (Spain)	380	10	Maximum values: TN-RE ≈ 87±1%. Biomass concentration ≈ 21.8±0.3 g m^{-2} d^{-1}; Lipid content (20.8±0.2%)	Arbid et al., 2013b
Secondary domestic	Flue gas	O: Cádiz (Spain)	330	4	Maximum values: TN-RE ≈ 95%; TP-RE ≈ 95%; Biomass productivity ≈35 g m^{-2} d^{-1}; Biomass composition: C (40.0±1.0% to 47.5±2.05%); N (3.2±0.1% to 4.2±0.2%); Lipids (19.7±0.7% to 25.7±0.9%)	Arbid et al., 2013
Diluted Centrates	Flue gas	O: Almería (Spain)	340	3	Production of microalgae at dilution rates < 30%; Biomass concentration of ≈ 0.48 g L^{-1} at 15% centrate dilution; Max TN-RE≈ 90%	Ledda et al., 2015
Fish farm	Pure CO_2	O: Vlissingen (The Netherlands)	40	Runs of 15 d	No P addition/ P addition: Biomass concentration ≈ 0.5 g L^{-1}/ ≈ 1.0 g L^{-1}; TN-RE ≈ 49.4%/ ≈ 95.7%; TP-RE ≈ 99.0%/ ≈99.7%	Michels et al., 2014

Table 5. Fundamentals, advantages and disadvantages of the most applied microalgal biomass harvesting techniques (De Godos et al., 2011; Barros et al., 2015; Christenson and Sims, 2011). BREf= Biomass Recovery Efficiency

PROCESS	METHOD	FUNDAMENTALS	ADVANTAGES	DISADVANTAGES
CHEMICAL	**Flocculation/Coagulation**	Addition of different flocculants/coagulants (chitosan, $AlCl_3$ or $(Fe_2SO_4)_3$) to promote the subsequent sedimentation of microalgae (BREf: ≈ 67-99%)	Simple and fast / Low energy requirements	High costs of chemical flocculants/ Adjustment of the pH of the treated WW
MECHANICAL	**Flotation**	Gas bubbles supplied to the cultivation broth provide the lifting force needed for microalgae flotation and separation. Commonly used in WW treatment preceded by coagulation/flocculation processes (BREf: ≈ 32-92%)	Feasible for large scale applications/ Low space requirements/ Short hydraulic retention times	Often needs a previous flocculation/coagulation step
	Centrifugation	Centrifugation of the algal broth (dewatering). Cost-effective for high value products (BREf: ≈ 90-96%)	Fast method/ High biomass recovery efficiencies/ Suitable for all microalgal species	Very expensive/ High energy requirements/ Possible cell damage due to high shear forces
	Filtration	Microalgae filtration through a membrane (dewatering) (BREf: ≈ 82-98%)	High biomass recovery efficiencies/ Separation of the shear sensitive species	Need for a previous coagulation/flocculation step/ Fouling or clogging of the membrane increases operating costs/ Regular cleaning of the membrane/ High cost of the membrane replacement and pumping
	Gravity sedimentation	Natural biomass settling. Optimum for low value products (BREf: ≈ 30-98%)	Simple and inexpensive	High HRT→ Possibility of biomass deterioration/ Low concentration of algae sludge/ Settling capacity dependent on microalgae species
	Electrical based methods	An electrical field is applied to the cultivation broth and the cells (negatively charged) are separated (BREf: ≈ 60-99%)	Applicable to a wide variety of microalgal species	High energy and investment costs/ Low experience available
BIOLOGICAL	**Auto and bioflocculation**	Autoflocculation as a consequence of the pH increase mediated by the microalgal photosynthesis (BREf: ≈ 90%)	Inexpensive/ Non toxic to microalgal biomass	Changes in cellular composition/ Possibility of microbiological contamination

b. Biofilm Photobioreactors

Algal-bacterial cells can be also confined within a matrix (enclosure method) or in a biofilm attached onto the photobioreactor's surface (non-enclosure method) (Posadas et al., 2013; Katarzyna et al., 2015). However, the high price of immobilization materials (e. g. carrageenan, chitosan and alginate) and their structural weakness during long term operation (particularly at high PO_4^{3-} concentrations) have promoted a major development of non-enclosure methods (Hoffmann, 1998; De Godos et al., 2009b; Muñoz et al., 2009).

Biofilm photobioreactors allow for an efficient carbon and nutrient removals from different WW such as secondary or tertiary domestic effluents, digested and raw manure effluents and industrial WW at low HRTs (Boelee et al., 2011; Zamalloa et al., 2013; Craggs et al; 1996; Kebede et al., 2004). This photobioreactor design prevents from biomass washout and offers microalgae protection against pollutant toxicity due to the diffusional gradients established within the biofilm (Hoffman, 1998). Microalgae can be harvested by scratching the biofilm surface in open algal turf scrubbers or by settling after biofilm detachment (Posadas et al., 2013; 2014b; Boeele et al., 2011). However, biofilm biomass harvesting frequency is still a critical issue during WW treatment, which must be optimized in each photobioreactor depending on its design and C, N and P loading rates since it ultimately determines biofilm thickness (Kesaano and Sims, 2014). Steady state thicknesses of the attached algal biomass in biofilm photobioreactors can vary from 130 μm to 4 mm, with a water layer thickness from 10 to 20 mm (Boelee et al., 2014). This thin algal-water layer implies high water evaporative rates per volume of photobioreactor and high temperatures due to the high volumetric liquid-gas mass transfer coefficient and the low thermal inertia, respectively (Murphy and Berberog, 2012). Another critical consideration is the availability of light due to the immobilization of high concentrations microalgae cells, which could eventually induce light limitation in the inner part of the biofilm and photoinhibition/photooxidation in the cells continuously exposed to solar irradiance (Schnurr et al., 2014). In this context, Schnurr et al. (2014) empirically determined that only the first few hundreds micrometres of microalgal biofilms receive enough light to be photosynthetically active. Mixing and CO_2/O_2 gas exchange are also crucial in biofilm photobioreactors, which is often carried out via an internal liquid recirculation flow of ≈5-20 cm s^{-1} (Kesaano and Sims, 2014; Wilkie and Mulbry, 2002). Currently, the main challenge of biofilm photobioreactors is their transition from bench and pilot scale to full-scale (Kesaano and Sims, 2014). The main configuration of algal-bacterial biofilm photobioreactors evaluated up to date is the algal turf scrubber (ATS), the enclosed algal-bacterial biofilm photobioreactor (EPBR) and the rotating algal-bacterial photobioreactor (RPBR) (Fig. 7).

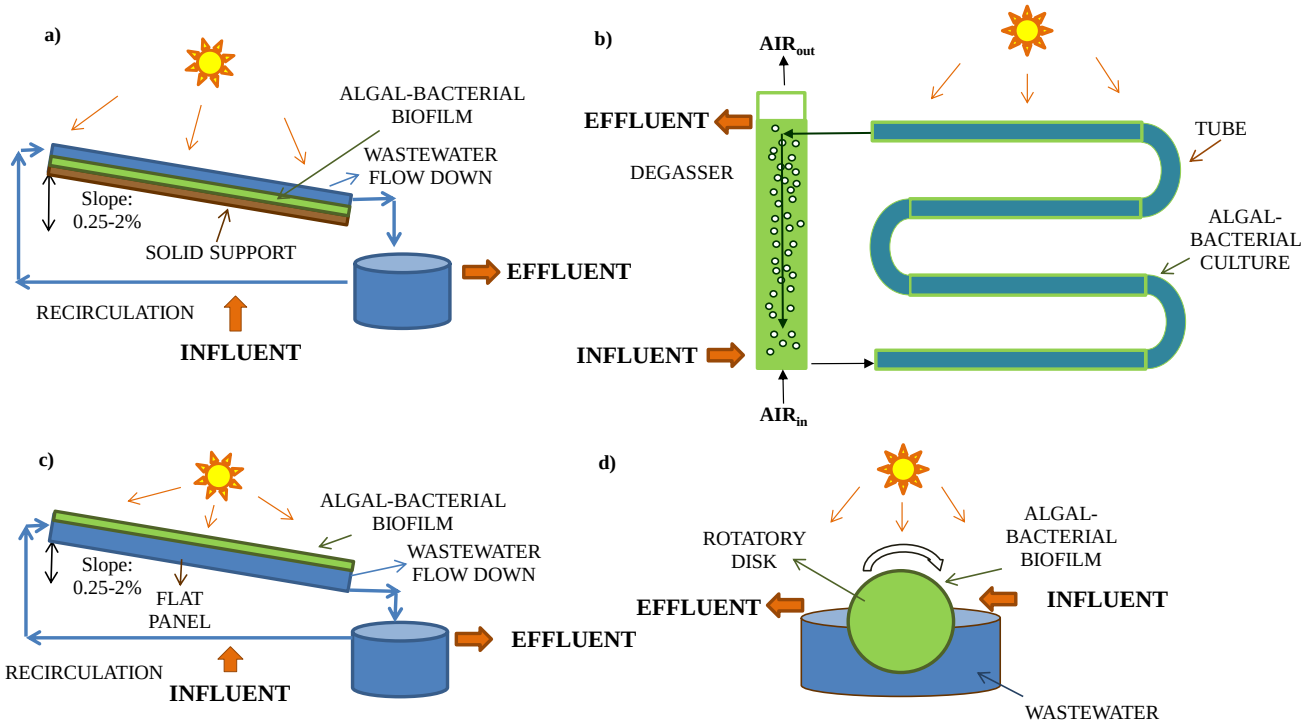


Figure 7. Different configurations of biofilm photobioreactors: **a)** Algal-turf scrubber (ATS) (open system); **b)** Tubular enclosed photobioreactor (TPBR); **c)** Flat plate enclosed photobioreactor (FPBR); **d)** Rotating algal-bacterial photobioreactor (RPBR) (open system).

ATSs are open photobioreactors where the growth of microalgae (mainly filamentous) and benthic bacteria occurs in the form of biofilm into an inclined plastic mesh (≈0.5-2% slope) and the wastewater flows downwards (Fig. 7a) (Alcántara et al., 2015c). The microbial community attached onto the liner in the ATSs is called periphyton and supports both carbon and nutrient removal from WW, biosorption of heavy metals and the photosynthetically mediated increase in pH and DO concentration (D'Aiuto et al., 2015). These photobioreactors were developed in the early 1980s by Dr. Walter Adey at the Smithsonian Institution in Washington (USA) (Adey et al., 2013). The effectiveness of the ATSs for carbon and nutrient removal has been successfully proven in aquacultural, agricultural, domestic and industrial WW (Table 6) (Posadas et al., 2013; Kangas and Mulbry, 2014; Alcántara et al., 2015c; Craggs et al., 1996). The main advantages of ATSs are their simple design and construction and their high biomass productivities (≈25-45 g m^{-2} d^{-1} when nutrients availability and irradiance are high) (Adey et al., 2013). Another important advantage is that the separation of treated wastewater and algal biomass can be easily carried out by scrapping or vacuuming the ATS surface (Posadas et al., 2013; 2014b; Adey et al., 2013; Mulbry et al., 2008). Posadas et al. (2013) identified their high water evaporation rates and high carbon and nitrogen stripping as the main disadvantages of these photobioreactors in terms of wastewater treatment.

EPBRs were designed to overcome the above mentioned disadvantages of ATS during agricultural, domestic and industrial wastewaters treatment (Posadas et al., 2014b; González et al., 2008b; De Godos et al., 2009b; Zamalloa et al., 2013; Muñoz et al., 2009). Tubular (Fig. 7b) and flat plate (Fig. 7c) biofilm photobioreactors are so far the only configuration of EPBRs evaluated for WW treatment (Alcántara et al., 2015c). In this particular photobioreactor configuration, the algal-bacterial biomass grows attached onto the inner part of the photobioreactor wall as a consequence of its natural capacity of adherence to the surfaces (Zamalloa et al., 2013). The main difference between EBPR in suspended or attached growth configurations is the use of inner particles that in a suspended system avoid the growth of the biomass into the surface, while in an attached system the absence of these particles aims the growth of the biomass onto the surface. De Godos et al. (2009b) recorded N and P removal efficiencies of 94-100% and 70-90%, respectively, during the treatment of centrifuged swine slurry in a 7 L tubular biofilm photobioreactor. Unfortunately, biomass clogging inside enclosed photobioreactors is likely to occur during the treatment of secondary domestic wastewater if not enough shear stress is provided by the recirculating cultivation broth (Posadas et al., 2014b). Despite the promising results obtained, the performance of EPBRs for WW treatment

should be further tested at large scale under outdoors conditions in order to elucidate the full potential of this photosynthetic biofilm technology (Table 7).

RPBRs are rotating cylinders with the algal-bacterial biofilm attached onto the external cylinder surface partially submerged in wastewater, which are alternatively rotated to expose the biofilm to the wastewater and the open atmosphere (Fig. 7d) (Christenson and Sims, 2012). Similarly to their biofilm photobioreactor counterparts, algal harvesting by scrapping constitutes an important economic advantage in this system (Gross et al., 2013). RPBRs are based on the conventional rotating disks used in WWTPs, where bacterial biofilms have shown an efficient organic matter removal at large scale. Despite RPBRs have been tested only at lab-scale mainly for tertiary wastewater treatment, recent studies have been focused on exploring optimal operation conditions to scale-up RPBRs (Gross et al., 2013) (Table 8).

Table 6. Results obtained during the treatment of wastewaters in ATSs. O=outdoors; I=laboratory conditions (indoor); RE= removal efficiency

Wastewater	Gas treatment	Illumination/City	Volume (L)	HRT (d)	Relevant information	Reference
Secondary domestic	---	O: Patterson (USA)	1021 m^2 of cultivation surface	Flow rate: 436-1226 m^3 d^{-1}	TN-RE ≈ 1.11±0.48 g m^{-2} d^{-1}; TP-RE ≈ 0.73±.28 g P m^{-2} d^{-1}; Max. Biomass productivity ≈ 35 g m^{-2} d^{-1}; Biomass composition: N (3.1%); P (2.1%)	Craggs et al., 1996
Diluted centrates and primary domestic	---	I: PAR ≈ 88±16 µmol m^{-2} s^{-1}/ light:dark cycles 16:8 h	31 (0.5 m^2 of cultivation surface)	3.1-10.4	TC-RE: ≥80%; TN-RE: 70±8%; TP-RE: 85±9%; Max. Biomass productivity ≈ 3.1 g m^{-2} d^{-1} : Evaporative rate: 0.5–6.7 L m^{-2} d^{-1}	Posadas et al., 2013
Secondary domestic	---	O: Glogow (Poland)	4 m^2 of cultivation surface	1-4	Max. TP-RE ≈ 97±1%; Biomass productivity ≈ 5.6 ± 1 g $m^{-2}d^{-1}$	Sukacova et al., 2015
Dairy manure	---	I: PAR ≈ 40-140 µmol m^{-2} s^{-1}/ light:dark cycles 16:8 h	1.86 m^2 of cultivation surface	≈ 20	COD-RE ≈ 77%-95%; TN-RE ≈ 39-62%; TP-RE ≈ 51-93%; Biomass composition: N (4.9–7.1%); P (1.5–2.1%); Max. Biomass productivity ≈5.5 g $m^{-2}d^{-1}$	Wilkie and Mulbry, 2002
Primary domestic	---	I: PAR ≈ 74±3 µmol m^{-2} s^{-1}/ light:dark cycles 16:8 h	31 (0.5 m^2 of cultivation surface)	5-10	Max. values: TOC-RE ≈ 89±2%; TN-RE ≈ 92±5%; TP-RE ≈ 96±2%; Max. Biomass productivity: ≈ 3.6±0.8 g m^{-2} d^{-1}; Evaporative rate ≈ 3.6±0.8 L m^{-2} d^{-1}	Posadas et al., 2014b
Dairy manure	---/ Pure CO_2	O: Maryland (USA)	30 m^2 of cultivation surface	Effluent of 3500 L d^{-1}	Max. biomass productivity ≈ 25 g $m^{-2}d^{-1}$; Biomass composition: N (7%); P (1%);No differences with CO_2 supply	Mulbry et al., 2008

Table 7. Results obtained during the treatment of wastewaters in EPBRs. O=outdoors; I=laboratory conditions (indoor); RE= removal efficiency.

Wastewater	Illumination / City	Volume (L)	HRT (d)	Relevant information	Reference
Primary domestic	O: Gent (Belgium)	5 L (0.5 m^2 of cultivation surface)	1	COD-RE ≈ 74%; TN-RE ≈ 67%; TP-RE ≈ 96%; TSS-RE ≈ 82%; Biomass productivity ≈ 2.5 g $m^{-2}d^{-1}$	Zamalloa et al., 2013
Swine manure	I: PAR ≈ 135 µmol m^{-2} s^{-1}/ light:dark cycles 24:0 h	4.9	10	COD-RE ≈ 75%; Max. NH_4^+-RE ≈ 99%, max. P_s-RE ≈ 86%	González et al., 2008b
Swine manure	I: PAR ≈ 135 µmol m^{-2} s^{-1}/ light:dark cycles 24:0 h	7.5	7	NH_4^+-RE ≈ 94-100%: P-PO_4^{3-} RE ≈70%-90%; Biomass retention: ≥92%; Max. pH ≈10.3	De Godos et al., 2009b
Primary domestic	I: PAR ≈ 74±3 µmol m^{-2} s^{-1}/ light:dark cycles 16:8 h	31 (0.5 m^2 of cultivation surface)	5-10	Max. values: TOC-RE ≈ 89±2%; TN-RE ≈ 35%: TP-RE ≈ 0%; ↓↓ Biomass productivity	Posadas et al., 2014b

Table 8. Results obtained during the treatment of wastewaters in RPBRs. O=outdoors; I=laboratory conditions (indoor); RE= removal efficiency

Wastewater	Illumination/City	Volume (L)	HRT (d)	Relevant information	Reference
Secondary domestic	I: PAR ≈ 170 µmol m^{-2} s^{-1}/ light:dark cycles 14:10 h	8/535	10	Average removal rates: TN ≈ 14.1 g $m^{-2}d^{-1}$; Ps ≈ 2.1 g m^{-2} d^{-1}; Max. biomass productivity ≈ 5 g m^{-2} d^{-1}; Efficient harvesting : biomass concentration→12-16% solids	Christenson and Sims, 2012
Synthetic petroleum hydrocarbon	I: 1000-1100 lux/ light:dark cycles 18:6 h	4 (0.83 m^2 of cultivation surface)	1-12 (semi-continuous with different loads of phenol)	Max. COD-RE ≈ 97%; TP-RE ≈ 97%; Tolerant limit of diesel concentration for microalgae: 0.08%;	Chavan and Mukherji, 2010
Synthetic acid mine drainage	I: PAR ≈ 189 µmol m^{-2} s^{-1}/ light:dark cycles 12:12 h	15	1	Max. heavy metals-RE: %Cu≈51%; %Ni≈47%; %Mn≈45%; %Zn≈35%; %Sb≈50%; %Se≈50%; %Co≈15	Orandi et al., 2012

1.5 Biomass composition and applications

Proteins (6-52%), lipids (5-77%), carbohydrates (5-82%) and ashes (5-15%) are the main microalgae constituents (Rebolloso Fuentes et al., 2000; Chisti, 2007; Serejo et al., 2015; Gómez et al., 2013; Zhu, 2015). Microalgae also represent a valuable feedstock for the production of key vitamins such as A, B_1 or B_6, and pigments such as chlorophyll (0.5-1.5%) and carotenoids (0.1-0.2% although *Dunaliella* can accumulate up to 14%) (Becker, 2007). This wide variability in microalgal biomass composition is based on the high diversity of microalgae species and their high metabolic plasticity (George et al., 2014; Zhu, 2015). For instance, the major fraction of the harvested biomass cultured without nutrient limitations is protein, which could achieve contents of up to 52% (Sialve et al., 2009). On the other hand, microalgae cultivation under nitrogen starvation conditions can boost lipid accumulation (Breuer et al., 2012). Thus, Toledo-Cervantes et al. (2013) recorded an increase in non polar lipids from 20 to 55.7% when *Scenedesmus obtusiusculus* was subjected to nitrogen limitation. Similarly, microalgae can accumulate carbohydrates under phosphorous limiting conditions (Margarites and Costas, 2014). For instance, Serejo et al. (2015) recorded a microalgae carbohydrate content of 81.8±1.3% when phosphorus limited the biodegradation process during the treatment of diluted anaerobic digested vinasse in a 180 L HRAP. However, microalgae cultivation coupled to WW treatment limits tailoring biomass composition via culture medium optimization, although microalgae composition has been reported to be influenced by WW composition (Posadas et al., 2015c). This high variability on microalgae chemical composition implies the need to characterize the biomass under each particular operation conditions (Zhu, 2015).

Chlorella, *Arthospira* (*Spirulina*) *platensis*, *Dunaliella*, *Haematoccus*, *Porphyridium* sp., *Nannochloropsis* and *Nostoc* are among microalgae species of industrial interest (Iwamoto, 2004; Hu, 2004; Ben-Amotz, 2004; Cysewski, 2004; Arad and Richmond, 2004; Zitelli et al., 2004; Danxiang et al., 2004). Currently, microalgae are mainly applied for human nutrition supplements and high value products (e. g. astaxanthin, β-carotene or ω-3) while their main uses under current research are the production of biofuels and biofertilizers and animal nutrition (poultry, pigs, ruminants and aquaculture) (Becker, 2004; Acién et al., 2014; Chisti, 2007; Romero García et al., 2012b). The production of bioplastics has also been suggested as alternative application of microalgae and it is currently under study based on the fact that microalgae can accumulate poly-β-hydroxybutyrates (PHBs) (up to intracellular level of 21.5% cell dry in *Nostoc Muscorum*) (Serejo et al., 2015; Zeller et al., 2013; Haase et al., 2012; Balaji et

al., 2013). In the context of WW treatment, the most straight-forward use of the harvested biomass might be as a feedstock for the production of commodities such as biofuels and biofertilizers (Acién et al., 2014).

1.5.1 Biofuels

a. Biodiesel

Numerous investigations have been focused on the production of biodiesel from microalgae based on their potential high lipid content (*Schizochytrium* sp. can accumulate until 77%) since the publication of the article *Biodiesel from microalgae* in 2007 by the professor Yusuf Chisti (Chisti, 2007). Several strategies, as the above mentioned nitrogen limitation, have been applied to trigger microalgae lipid accumulation. Likewise, innovative downstream processes have been concomitantly developed to extract the lipid content from microalgae, *in-situ* transesterification of wet biomass with methanol being one of the most widely applied (Toledo-Cervantes et al., 2013; Cerón-García et al., 2013). However, there are still some constraints to produce biodiesel from the harvested biomass obtained from wastewater treatment due to their low lipid content (2-23%), which makes biodiesel production non-sustainable from an economic and environmental view point (Posadas et al., 2016; Sepúlveda et al., 2015; Serejo et al., 2015; Gómez et al., 2013).

b. Bioethanol

The high carbohydrate content recorded in the harvested biomass obtained during WW treatment (30-82%) has raised interest in the production of bioethanol (Posadas et al., 2015; Serejo et al., 2015; Cea-Barcia et al., 2014). However, the industrial production of bioethanol from microalgae is still under development, the effectiveness of the pre-treatments needed for microalgae disruption and hydrolysis of the complex carbohydrates into simple sugars being one of the limiting steps of this biotechnological process (Hernández et al., 2015).

c. Biogas

The anaerobic digestion of the microalgae biomass generated during WW treatment has been investigated since the late 1950s (Metcalf and Eddy, 2003). Biogas production is species-specific and, similarly to bioethanol production, numerous pre-treatments have been developed to disrupt the strong cell walls of microalgae and make the intracellular content available to the anaerobic community (Ramos Suárez et al., 2014; Alzate et al., 2014).

Alcántara et al. (2013) recorded a recovery of energy of 48% and 61% from the chemical energy fixed during photoautothopic and mixotrophic microalgae growth, respectively. On the other hand, the high protein content of the microalgal biomass can inhibit the activity of the anaerobic community as a result of the high NH_4^+ concentrations (Ramos Suárez et al., 2014; Olsson et al., 2014).

d. Biohydrogen

The production of biohydrogen from microalgae can be carried out by biophotolysis (direct or indirect photolysis) or by catabolism of endogenous substrate (Show and Lee, 2014). Currently, the production and the molecular pathways above mentioned are under investigation in order to optimize the process (Oncel et al., 2015).

The production of only one type of biofuel from microalgae is not an economically or environmentally sustainable alternative based on the above mentioned disadvantages of the individual bioprocesses. In this context, the implementation of a biorefinery approach is crucial for a cost-effective and sustainable valorization of the algal-bacterial biomass produced during WW treatment (Zhu et al., 2015; Acién et al., 2014). A biorefinery consists of separation of the different components present in microalgal biomass (primary biorefinery) and their subsequent downstream (secondary biorefinery) to obtain different biofuels or bioproducts (Zhu et al., 2015).

1.5.2 Biofertilizers

The use of microalgae cultivated in WW as biofertilizers has been repeatedly proposed based on their high protein content (Romero García et al., 2012b; Ördög et al., 2004; Sepúlveda et al., 2015). In this regard, Romero García et al. (2012) developed an enzymatic process to produce a concentrated free-aminoacid solution from microalgal biomass that is currently under commercialization.

1.6 Algal-bacterial processes for the simultaneous wastewater and gas treatment

Most wastewaters often present a low C/N/P ratio compared to the ratio 100/18/2 recorded in the harvested biomass, which entails the occurrence of a carbon limitation during microalgae-based WW treatment (Posadas et al., 2014; Park and Craggs, 2010). In this context, the

additional supply of biogas or flue gas to the cultivation medium (Fig. 8), could increase the availability of inorganic carbon in the cultivation medium and therefore, biomass productivity and nutrient recovery from WW (Arbid et al., 2013). CO_2 supply into the cultivation broth also contributes to pH control (Acién et al., 2012; Arbid et al., 2013). In this context, algal-bacterial symbiosis can support a combined wastewater treatment and biogas upgrading or flue gas purification (Serejo et al., 2015; Bahr et al., 2014) (Fig. 8).

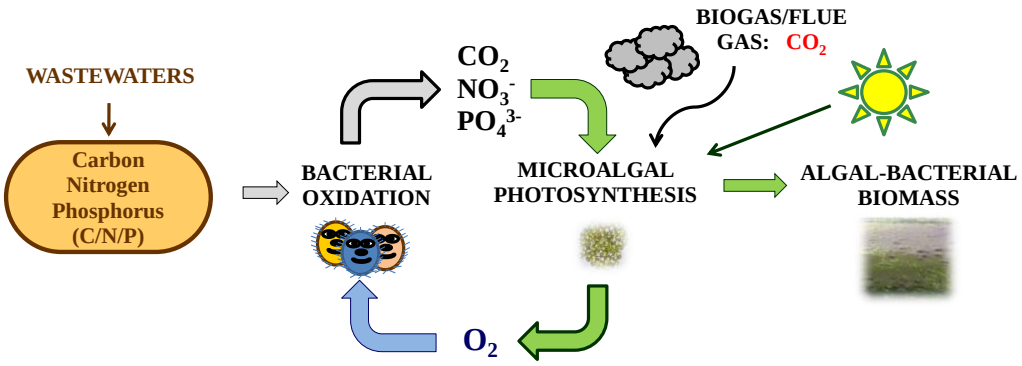


Figure 8. Algal-bacterial synergetic interactions during the simultaneous treatment of wastewater and CO_2 from biogas or flue gas.

1.6.1 Wastewater treatment coupled with biogas upgrading

Biogas, which is produced via anaerobic digestion of organic substrate, constitutes a biofuel able to reduce the current fossil fuel dependence of our society (Muñoz et al., 2015). The total biogas primary energy produced during 2013 in the European Union accounted for 13.4 Mtoe, which corresponded to an electricity generation of 52.3 TWh (EurObserv'ER, 2014). Biogas is produced in WWTPs (sludge anaerobic digestion), in landfills and during the anaerobic digestion of agricultural or agro-industrial organic wastes (Ryckebosch et al., 2011). Despite its chemical composition can vary depending on the nature of the organic substrates and the operational conditions during anaerobic digestion, raw biogas is mainly composed of CH_4 (40-75%), CO_2 (15-60%), H_2O (5-10%), N_2 (0-2%), H_2S (0.005-2%), O_2 (0-1%), NH_3 (<1%), halogenated hydrocarbons (<0.6%), CO (<0.6%) and siloxanes (0-0.02%) (Ryckebosch et al., 2011; Muñoz et al., 2015). Biogas can be used for industrial and domestic heating in kitchen stoves, as a substrate in fuel cells, as a fuel for the combined production of electricity and heat, as a feedstock to produce fine and bulk chemicals, as a vehicle fuel or it can be injected into the natural gas grid for industrial or domestic uses according to the European Directive 2003/55/EC (Bauer et al., 2013; Wellinger and Lindberg, 1999; Weiland, 2010). However, the efficient use of biogas in the two latter applications requires a previous upgrading to

"biomethane" in order to achieve a CH_4 content of 95-97% and 1-3% of CO_2 (Bauer et al., 2013; Ryckebosch et al., 2011). In this context, countries like Germany, Sweden, Spain and Switzerland have defined quality standards for biomethane injection into their natural gas grids (Weiland, 2010; Persson et al., 2006). For instance, the purity of CH_4 in the upgraded biogas must be higher than 95% for injection into the grids, with a CO_2 and O_2 contents lower than 2% and 0.3%, respectively, according to the Spanish legislation (BOE, 2013). Likewise, the H_2S levels required for biomethane injection into natural gas grids and for use as a vehicle fuel should be lower than 5 mg m^{-3} (0.0004%) (Bailón and Hinge, 2012). In this regard, the removal of CO_2 and H_2S from raw biogas is needed since it entails a decrease in biogas transportation and compression costs, an increase of its specific calorific value and a reduction of the toxicity, bad odors and corrosion in pipelines and engines (Serejo et al., 2015).

Conventional technologies for CO_2 removal from biogas are based on physical/chemical processes such as scrubbing with water, organic solvents or chemical solutions, membrane separation, pressure swing adsorption, vacuum swing adsorption and cryogenic CO_2 separation (Muñoz et al., 2015; Ryckebosch et al., 2011). Despite these technologies are the most commonly applied at large scale based on their commercial availability (Bauer et al., 2013), physical/chemical technologies present some disadvantages such as a low membranes selectivity, high investment and operating costs along with high environmental impact (Ryckebosch et al., 2011; Bailón and Hinge, 2012; Molino et al., 2013). On the other hand, biotechnologies for CO_2 removal from biogas such as chemoautotrophic, photosynthetic or enzymatic (immobilized enzyme carbonic anhydrase) processes exhibit low environmental impact and low operating costs. Unfortunately, they have been mainly tested at laboratory and pilot scale (Ryckebosch et al., Muñoz et al., 2015). Similarly, technologies for H_2S removal from biogas can be classified into physical/chemical (*in-situ* chemical precipitation, adsorption, absorption and membrane separation) and biological (biofiltration, *in-situ* microaerobic H_2S removal and algal-bacterial processes) (Ryckebosch et al. 2011, Muñoz et al., 2015; Bahr et al., 2014; Ramos et al., 2013). Biological techniques for H_2S removal are gradually replacing their physical/chemical counterparts as a result of their comparable removal efficiencies and their lower operating costs and environmental impacts (Muñoz et al., 2015).

Physical/chemical technologies such as water/chemical scrubbing and membrane separation allow for a simultaneous removal of CO_2 and H_2S at the expenses of high operation costs (Tippayawong and Thanompongchart, 2010; Ryckebosch et al. 2011; Bahr et al., 2014). In this context, microalgae-based processes allow for a simultaneous CO_2 and H_2S removals from

biogas under an innovative, environmentally friendly and low-cost operation (Posadas et al., 2015c). This biotechnology is based on the simultaneous CO_2 consumption by microalgae mediated by photosynthesis and the oxidation of H_2S to SO_4^{2-} by sulfur oxidizing bacteria (or chemical oxidation) using the O_2 produced by microalgae (Bahr et al., 2014; Muñoz et al., 2015). The economic feasibility and sustainability of this process can be enhanced if microalgae are cultivated in wastewaters, which would entail a simultaneous biogas upgrading and wastewater treatment (Fig. 9) (Serejo et al., 2015).

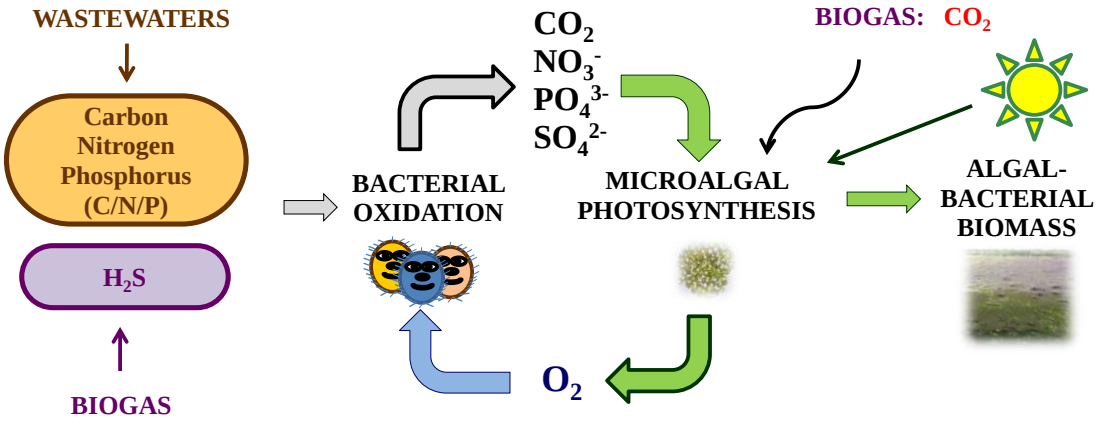


Figure 9. Algal-bacterial synergistic interactions during the simultaneous wastewater treatment and biogas upgrading.

The integration of these processes has been addressed in the past. For instance, Conde et al. (1993) coupled piggery wastewater treatment and biogas upgrading in a 15 L HRAP coupled with an internal biogas absorption column (namely BIOLIFT). The authors obtained a final biogas composition of: 88-97% CH_4, 2.5-11.5% CO_2 and less than 0.5% of H_2S (from an initial composition of 55-71% CH_4, 44-48% CO_2 and less than 1% of H_2S). Similarly, Zhao et al. (2013) obtained a maximum CH_4 purity of 92.74±3.56% under culture irradiances of 1200-1600 µmol m^{-2} s^{-1} during the simultaneous treatment of digestate and biogas in 96 1-L bag-photobioreactors. Bahr et al. (2014) evaluated the simultaneous treatment of diluted centrate and biogas upgrading in a 180 L HRAP operated at 23 d of HRT coupled with an absorption column of 2.5 L. The results showed a final CO_2 removal efficiency of 40% concomitant with a complete H_2S removal regardless of the operational conditions, which was favored by its higher solubility in the aqueous phase (adimensional Henry's constant of 2.44 for H_2S compared to 0.83 for CO_2 (Sander, 1999)) and the high DO concentrations in the cultivation broth mediated by photosynthetic activity. In a similar experimental set-up, Posadas et al. (2016) recorded CO_2 removals from synthetic biogas of 99% and O_2 concentrations in the upgraded biogas of ≈ 20% during the treatment of diluted centrates, while Serejo et al. (2015)

recorded CO_2 and H_2S removals of ≈ 80% and 100%, respectively, with O_2 concentrations in the upgraded biomethane ranging from 2±1% to 1±0% during the treatment of diluted anaerobically digested vinasse. Despite this significant reduction in the concentration of O_2 in the upgraded biogas between both investigations, the final O_2 concentration reported by Serejo et al. (2015) was still higher than the regulations established by BOE (2013) to inject the biomethane into natural gas grids. These high O_2 concentrations in the biomethane were caused by the desorption of the photosynthetically produced O_2 concentrations from the recycling algal cultivation broth, which severely challenges the application of this novel biotechnology. In this context, Posadas et al (2015c) evaluated several operational strategies to minimize the O_2 concentration in the final biomethane in a similar experimental design treating anaerobically digested vinasse. The authors obtained a maximum CO_2 and H_2S removals of 72±1% and 100±0%, respectively, along with O_2 levels of 0.7±0.2%, very close to the compliance with the requirements for injection of biomethane in natural gas networks. However, the purity of CH_4 was 81±2% as a result of the high N_2 concentrations (≈6-8%), which was also desorbed from the cultivation broth. Similar N_2 contamination issues have been recorded in water scrubbing technologies, which requires further investigations (Bauer et al., 2013).

1.6.2 Wastewater treatment coupled with flue gas purification

The total annual anthropogenic CO_2 emissions have increased from 22 to 33 Gt from 1990 to 2010, and they are expected to reach 41 Gt by 2030 although the target worldwide emission was set at 26 Gt (World Bank, 2014; United Nations, 2015). These high emissions cause important environmental problems such as an increase in the global warming effect and a modification of the pH in the oceans, which severely affects marine ecosystems (Hunter, 2007). The combustion of fossil fuels constitutes 93.5% of the total CO_2 emissions, which contain CO_2 concentrations ranging from 5 to 20% (EPA, 2015; Arbid et al., 2013; Warmuzinski et al., 2015; Raeesossadati et al., 2014). In this context, the development and implementation of technologies for the capture of CO_2 from industrial emissions is mandatory according to the European Directive 2009/31/EC.

Scrubbing with alkaline sorbents and cryogenic separation are among the conventional technologies for CO_2 removal from flue gases (Granite and O´Brien, 2005). Novel techniques such as electrochemical, membrane, enzymatic, photosynthetic, catalytic routes and chemical looping combustion for CO_2 separation or conversion exhibit lower operating costs than their conventional counterparts (Granite and O´Brien, 2005; Warmuzinski et al., 2015). However,

only the biological techniques present a low environmental impact. In this regard, photosynthetic CO_2 capture processes supported by microalgae in photobioreactors allows for the removal of CO_2 in an environmentally friendly way (Raeesossadati et al., 2014). In microalgae-based processes, the flue gases are sparged into the cultivation broth and CO_2 is transferred from the gas to the liquid phase (Sander, 1999). The dissolved CO_2 is then consumed by microalgae during photosynthesis in the presence of light. Therefore, the C-CO_2 from flue gas is recovered as a valuable algal biomass, which can be further valorized (Raeesossadati et al., 2014). The economic and environmental sustainability of the process can be significantly improved when microalgae cultivation is supported by a free nutrients and water source such as wastewaters (Park and Cragss, 2010).

Thus, Arbid et al. (2013) integrated flue gas purification from a 1600 MW combined cycle plant and tertiary domestic wastewater treatment in two HRAPs (one with sump, 593 L, and other without sump, 533 L) and in a tubular airlift photobioreactor of 330 L under outdoors conditions in Arcos de la Frontera (Cádiz, Spain). These authors concluded that CO_2 addition from flue gas increased both biomass productivity and the removal of nitrogen and phosphorus from wastewater. Similarly, Posadas et al. (2015) coupled secondary domestic wastewater treatment and flue gas purification in three outdoors HRAPs located in Almería (Spain) with volumes ranging from 700 L (without sump) to 850 (sump of 150 L). However, no increase in biomass productivity or nutrient removals efficiency was recorded during flue gas sparging due to the high contribution of CO_2 removal by stripping and the fact that the process was limited by light supply. De Godos et al. (2010) evaluated the influence of flue gas sparging during the treatment of diluted piggery effluents in two outdoors HRAPs of 465 L operated at 10 d of HRT in Valladolid (Spain). This study concluded that assimilation of the CO_2 transferred to the liquid phase would only occur under inorganic carbon limitation and will never take place under light, or nutrient limiting conditions. The integration of tertiary domestic wastewater treatment and flue gas purification at real scale is the main objective of the European Project All GAS, which has been conducted by AQUALIA since 2012. In this project, a total HRAP cultivation surface of 1000 m^2 is under construction for the treatment of the WW from Chiclana de la Frontera (Cádiz, Spain) coupled to CO_2 supplementation from flue gas from the olive pits combustion, which will be also used to control the pH of the cultivation broth (All-gas, 2013; De Godos et al., 2016).

1.7 Economic and environmental sustainability of algal-bacterial processes

1.7.1 Costs and integration in conventional WWTPs

HRAPs are the preferred photobioreactor configuration for WW treatment based on their low mixing costs (0.1 € L^{-1} WW treated) compared to TPBRs (1.6 € L^{-1} WW treated) and flat plate photobioreactors (3.9 € L^{-1} WW treated) (Acién et al., 2012; Norsker et al., 2011). Thus, different configurations for the integration of HRAPs in conventional WWTPs have been proposed during secondary and tertiary WW treatment (Steele et al., 2014). In this context, HRAPs require large areas for implementation as a result of their low photosynthetic efficiency, but are energy-efficient based on their low energy consumption for mixing, which would ultimately determine the size of the population to be served (Posadas et al., 2016b).

The removal of fixed suspended solids (FSS) in a conventional grit settler would provide higher loads of biodegradable COD for complete nutrient removal in algal-bacterial processes than the removal of part of bCOD during primary settling (Posadas et al., 2016b; Park and Craggs, 2011). Therefore, any HRAP designed for complete N or P removal from domestic WW *de facto* would provide free and environmental benign capacity for biodegradable COD removal (Posadas et al., 2016b). In this context, HRAPs should be more preferentially applied for secondary domestic WW instead of for tertiary WW, which would reduce an operation unit in conventional WWTPs (Posadas et al., 2016b). Likewise, and despite the positive energy balance of anaerobic digestion of the harvested biomass in HRAPs (extra 0.5 kWh m^{-3} WW treated), the management of the generated digestate implies the need to propose alternative uses of this biomass for economic integration in the WWTPs without the drawback of liquid effluents management (De Godos et al., 2016). In this context, Posadas et al. (2016b) proposed the use of solar drying for an efficient storage and biosolids transportation and reuse as biofertilizer.

1.7.2 Energy consumption and greenhouse gas (GHG) emissions

HRAPs constitute a favorable alternative to conventional processes such as activated sludge from an energy consumption viewpoint based on their low required energy for mixing (0.023 kWh m^{-3} WW treated in HRAPs compared to 0.33-0.62 kWh m^{-3} WW treated in activated sludge) (Alcántara et al., 2015c). Therefore, considering an electricity generation carbon footprint of 362 g CO_2 kWh^{-1} (EU) (IPCC, 2014), HRAPs would decrease the carbon footprint from ≈119-224 g CO_2 m^{-3} WW treated (activated sludge) to 8.3 g CO_2 m^{-3} WW treated.

Besides this reduction in CO_2 emissions, N_2O (a GHG with a 310 times higher global warming potential) emissions in HRAPs would also decrease from ≈ 0.01-6.6% N-N_2O kg N_{input}^{-1} in conventional activated sludge processes (depending on the configuration) to ≈ 0.047% N-N_2O kg N_{input}^{-1} (ICheme Metrics Sustainability, 2014; Kampschreur et al., 2009; Alcántara et al., 2015b). In this context, N-NH_3 volatilization, which is one the main nitrogen removal mechanisms in HRAPs, causes indirect N_2O emissions (conversion factor of 0.01 Kg N-N_2O Kg N-NH_3^{-1}) (IPCC, 2006; García et al., 2000). Hence, nitrification activity would contribute to reduce the overall carbon footprint of HRAPs by conversion NH_4^+ into NO_3^-, which would prevent ammonia volatilization. This fact will ultimately increase biomass productivity (Posadas et al., 2015).

1.7.3 Water footprint

The water evaporation (expressed as m^3 of water evaporated per m^3 of water treated) is directly proportional to the HRT and can compromise the environmental sustainability of the process (Guieysse et al., 2013; Alcántara et al., 2015c). Thus, Posadas et al. (2016b) estimated a maximum water evaporation of 6.4% in a 0.25 cm depth HRAP located in a temperate area and operated at 7 d of HRT. However, Guieysse et al. (2013) estimated a water evaporation of 15% in a HRAP located in Arizona at the above mentioned operational conditions. This water evaporations can represent up to 40 years of rainfall equivalent in Arizona, which challenges the application of HRAPs in areas with water scarcity (considering also the deterioration in the quality of the treated effluent) (Guieysse et al., 2013). Therefore, a further optimization of the operational strategies is required to decrease the water losses by evaporation, which constitutes the main responsible of water footprint in HRAPs (Alcántara et al., 2015c).

1.7.4 Land use

The land use of HRAPs can range from 5 to 13 m^2 $P.E.^{-1}$ (Alcántara et al., 2015c; Posadas et al., 2016b). These values, even the lowest reported, restrict the implementation of HRAPs to small-medium communities (Steele et al., 2014). However, due to the fact that cost of microalgae-based WWT is mainly driven by the cost of land, the initial investment is not lost as the land value would remain (or even increase in the long term) (Alcántara et al., 2015c). Therefore, and contrary to conventional WWTPs where the main investment costs are mechanical, electrical and civil equipments which are depreciated in the long term, algal-bacterial processes would not entail a loss of the initial investment.

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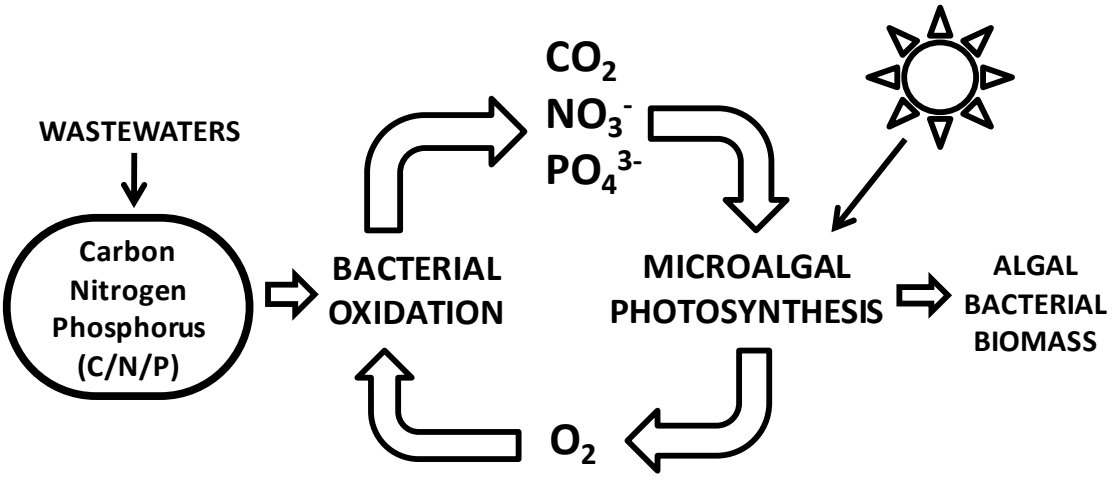
Aims and scope

Chapter 2



2.1 Justification of the thesis

The exponential increase of human population during the past century has resulted in the production of large amounts of WW, whose uncontrolled disposal has caused severe episodes of environmental pollution such as eutrophication and oxygen depletion in lakes and rivers. Despite the availability of physical/chemical and biological technologies for WW treatment since the earliest 1900s, there is still a lack of low-cost, environmentally friendly and sustainable technologies for the treatment of domestic and industrial WW. In this context, algal-bacterial processes have emerged as a potential alternative to conventional technologies. This bioprocess allows energy savings in aeration compared to conventional activated sludge WWTPs as a result of the free process oxygenation by microalgae during photosynthesis and compared to anaerobic processes due the enhanced nutrient removal supported by the dual heterotrophic-autotrophic algal-metabolism. In addition, the algal-bacterial biomass harvested from WW treatment could be used as a valuable feedstock for the production of biofuels and/or biofertilizers. Another important advantage of algal-bacterial processes derives from the possibility to integrate WW treatment with biogas upgrading or flue gas treatment, which contributes to mitigate GHG emissions and to enhance nutrient recovery from WW. However, and despite all these advantages, some limitations must be overcome prior scaling-up of microalgae-based WW treatment processes. For instance, the influence of the composition of the WW on the treatment performance is still unclear in algal-bacterial processes. Likewise, biomass harvesting constitutes one of the main economic bottlenecks for the full-scale implementation of this biotechnology, which requires the development of innovative harvesting strategies. The dynamics of microalgae and bacteria populations during WW treatment are also unknown, while the optimization of the simultaneous WW and gas treatment should be further evaluated at pilot scale. Finally, the viability of the application of microalgal biotechnology in a conventional WWTP should be evaluated in terms of efficient use of land and energy, while meeting the current Directive for WW treatment discharge into the environment with an effective biosolid management. Thus, more research focused on the optimization of this biotechnology should be carried out in order to overcome the above mentioned limitations and move microalgae-based processes from a promising lab-scale process to a sustainable full scale technology.

2.2 Main objectives

The overall objective of the present thesis was to determine the potential, limitations and challenges of algal-bacterial processes for an optimum WW treatment under different

photobioreactor configurations and operational conditions prior to technology scale-up. More specifically, the individual goals to achieve this overall objective were:

1. The study of the influence of the C/N/P ratio, dilution and inhibitory parameters of the WW on its final biodegradability in algal-bacterial systems in terms of carbon and nutrient removal efficiencies.
2. The evaluation of innovative photobioreactor designs to support a low cost biomass harvesting.
3. A comparative evaluation of conventional and emerging photobioreactor configurations for the treatment of multiple wastewaters.
4. Characterization of the dynamics of microalgae and bacteria populations present in the photobioreactors during WW treatment.
5. Optimization of the simultaneous biogas upgrading and WW treatment in algal-bacterial photobioreactors.
6. Integration of flue gas treatment and secondary domestic WW treatment in algal-bacterial systems.
7. Determination of the influence of pH and CO_2 source on the performance of open photobioreactors during secondary domestic WW treatment.
8. Evaluation of the integration of HRAPs within a full treatment system to efficiently using land, energy and water while meeting stringent requirements for nutrient removal and biosolid management.

2.3 Development of the thesis

In the present thesis, all experimental and literature review work was focused on the achievement of the main objective and on the evaluation of the potential application of this biotechnology at full scale.

In order to fulfill the first objective, five different agroindustrial wastewaters (potato processing WW (PW), fish processing WW (FW), animal feed production WW (MW), coffee manufacturing WW (CW) and yeast production WW (YW)) were chosen as representatives of the agroindustrial sector considering the high variability of these kind of wastewaters (**Chapter 3**).

Different designs of algal-bacterial biofilm photobioreactors were evaluated in order to enhance the harvestability of the biomass produced during WW treatment (**Chapters 4 and 5**).

Similarly, biomass settling in suspended growth HRAPs was also assessed to determine its cost effectiveness at large scale (**Chapters 6-7**).

The performance of several photobioreactors configurations to treat different wastewaters was evaluated in order to comply with the third objective, (**Chapters 4-8**). Thus, the performance of an algal-bacterial and a bacterial biofilm photobioreactors was compared during centrate and secondary domestic WW treatment (**Chapter 4**). Likewise, an open and an enclosed algal-bacterial biofilm photobioreactors were comparatively evaluated during secondary domestic WW treatment (**Chapter 5**). An outdoors HRAP of 180 L treating fish farm wastewater in Valladolid (Spain) (**Chapter 6**) and a similar indoor photobioreactor treating both centrate (**Chapter 7.1**) and anaerobically digested vinasse and raw vinasse (**Chapter 7.2**) were also evaluated. Finally, three HRAPs with volumes ranging from 700 to 850 L were assessed during secondary domestic WW treatment under outdoors conditions in the facilities of Las Palmerillas (Almería, Spain) (**Chapter 8**). The most relevant information about the influence of the environmental conditions on process performance was obtained in the experimental work conducted outdoors (**Chapters 6 and 8**).

The characterization of the structure of microalgae population was carried out in the comparative evaluation of the performance of an open and enclosed 31 L algal-bacterial photobioreactors treating secondary domestic WW at laboratory conditions (**Chapter 5**) and during the simultaneous biogas upgrading and centrate treatment in a indoor 180 L HRAP (**Chapter 7.1**). On the other hand, the characterization of both microalgae and bacteria population was carried in an indoor 180 L HRAP during anaerobically digested vinasse and raw vinasse treatment coupled with biogas upgrading (**Chapter 7.2**).

In order to fulfill objective 5, the treatment of centrate, anaerobically digested vinasse and raw vinasse in an indoor 180 L HRAP was coupled with biogas upgrading in an external 2.5 L absorption column (**Chapter 7**). **Chapter 7.1** was focused on the maximization of CO_2 removal from a synthetic biogas while **Chapter 7.2** evaluated different operational strategies to minimize the O_2 content in the upgraded biogas.

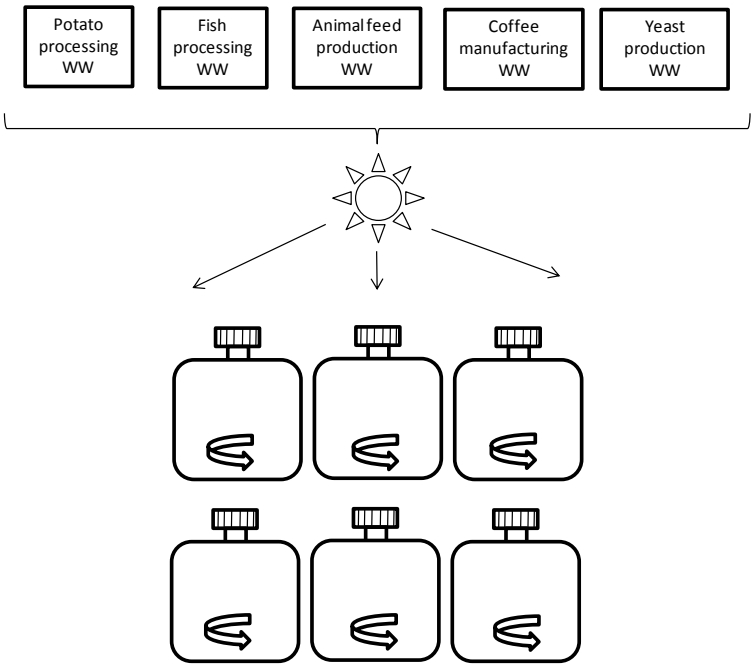
Objectives 6 and 7 were studied in **Chapter 8** in the three outdoors photobioreactors at pilot scale (from 700 to 850 L) under different seasonal conditions. During this research, domestic WW treatment was integrated with flue gas treatment under different pH values (from 7 to 9) to assess the influence of pH on WW treatment.

Biomass valorization was evaluated in several chapters. An analysis of the elemental composition of the biomass was carried in all experimental studies, while the macromolecular composition was only analyzed in **Chapter 8** to determine its potential valorization based on its fraction of carbohydrates, lipids, proteins and ash. Likewise, different nutrient starvation strategies were applied in **Chapter 7.1** to increase biomass lipid content during the simultaneous treatment of centrate and biogas upgrading. Finally, **Chapter 9** discussed the different applications of the harvested biomass (anaerobic digestion or solar drying prior to biomass use as biofertilizer) within a full integration of HRAPs in conventional WWTPs. **Chapter 9** also evaluated the required unit operations for algal-bacterial processes to support an efficient use of land, energy and water while optimizing biosolid management during wastewater treatment.

Microalgae-based agro-industrial wastewater treatment: a preliminary screening of biodegradability

Posadas E., Bochon S., Coca M., García-González M. C., García-Encina P. A., Muñoz R. (2014), J. Appl. Phycol. 26: 2335–2345.

Chapter 3



ALGAL-BACTERIAL SYMBIOSIS: BIODEGRADATION

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Microalgae-based agro-industrial wastewater treatment: a preliminary screening of biodegradability

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Abstract The potential of algal–bacterial symbiosis for the removal of carbon, nitrogen and phosphorus from five agro-industrial wastewaters was investigated in enclosed batch biodegradation tests using a mixed microalgae consortium and activated sludge as model microorganisms. The target wastewaters were obtained from potato processing (PW), fish processing (FW), animal feed production (MW), coffee manufacturing (CW) and yeast production (YW). The initial C/N/P ratio of the agro-industrial wastewater was correlated with its biodegradability. Thus, the highest removals of total organic carbon (TOC) and nitrogen were recorded in two fold diluted FW (64±2 % and 85±1 %, respectively), while the maximum P-PO_4^{3-} removal achieved was 89±1 % in undiluted PW. The biodegradable TOC was in most cases the limiting component in the treatment of the wastewaters evaluated. This study confirmed the potential of coupling carbon and nutrient recovery from agro-industrial effluents with the production of a valuable algal–bacterial biomass, despite their poor biodegradability.

Keywords Algal–bacterial symbiosis · Agro-industrial wastewaters · Carbon and nutrient removal · Photosynthetic oxygenation

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Introduction

Large volumes of wastewaters from industries processing agricultural and livestock raw materials are annually disposed to aquatic ecosystems worldwide as a result of the increasing food demand (Dareioti et al. 2009; Bhatnagar and Sillanpää 2010). In Spain, the total estimated volume of food-processing industry wastewaters produced in 2008 was 190,000,000 m^3 (Eurostat 2008). These agro-industrial effluents are mainly characterized by a high concentration of organic matter, nitrogen and phosphorus, and a variable pH (Drogui et al. 2008). Both the flow rate and characteristics of these wastewaters are industry specific and can vary significantly throughout the year because of the seasonal nature of the raw material processing (Dareioti et al. 2009). The uncontrolled disposal of such effluents in natural water bodies often results in surface and groundwater contamination and other environmental problems such as eutrophication and ecosystem imbalance (Drogui et al. 2008). Therefore, the development of cost-effective and environmentally friendly methods for the treatment of agro-industrial effluents is mandatory.

Although anaerobic digestion constitutes one of the most commonly used processes for agro-industrial wastewater treatment, its performance is often limited by poor nutrient removal (Rovirosa et al. 1995; Wilkie and Mulbry 2002). On the other hand, while activated sludge processes require a high-energy input for mechanical aeration, physical/chemical technologies such as adsorption, coagulation–flocculation or ion exchange involve prohibitive operating costs, which could compromise the economic viability of the agro-industries (González et al. 2008; Bhatnagar and Sillanpää 2010). In this context, microalgae-based treatment can overcome these limitations by supporting an in situ oxygen production via photosynthesis and nutrient removal via assimilation into the algal–bacterial biomass in a simple and economic process (De Godos et al. 2009). Thus, in the presence of sunlight,

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microalgae consume the CO_2 released during the bacterial mineralization of the organic matter and, in turn, produce the O_2 required by bacteria for the mineralization and NH_4^+ oxidation (Oswald 1988; Muñoz et al. 2005). This technology generates large amounts of residual microalgae biomass which constitutes a valuable feedstock for renewable energy production (Rawat et al. 2011; Rusten and Sahu 2011).

The first studies based on microalgal–bacterial symbiosis were carried out in California in the mid-1950s for the treatment of domestic wastewaters in high-rate algal ponds (HRAPs) (Oswald 1988), and recent studies have extended its application to industrial and livestock effluents (Muñoz and Guieysse 2006; González et al. 2011). However, little attention has been given to the treatment of agro-industrial wastewaters in microalgal–bacterial photobioreactors, despite their relevance. In this context, González et al. (1997) revealed the potential of microalgae-based systems for the removal of ammonia and phosphorus in agro-industrial wastewaters using the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. Nevertheless, due to the large variability in the characteristics of agro-industrial wastewaters, a systematic evaluation of the performance of this technology on each specific wastewater is needed to confirm its cost-effectiveness and to determine both the maximum biodegradation potential and limitations. This need was stressed in a recent study by Bahr et al. (2011), who reported that pollutant biodegradation in algal–bacterial systems was intrinsically linked to the carbon oxidation-reduction state.

This study systematically evaluated the potential and limitations of microalgal–bacterial symbiosis for the removal of carbon, nitrogen and phosphorus from five representative agro-industrial effluents in Castilla y León (Spain): potato processing, fish processing, animal feed production, lyophilized coffee manufacturing and yeast production. A mixed microalgae consortium from a HRAP in symbiosis with activated sludge was used as a model algal–bacterial consortium. Special attention was given to the elucidation of the parameters limiting the biodegradation process for each agro-industrial wastewater and to the carbon and nitrogen biomass content in each biodegradability test (the latter being relevant in the optimization of assimilatory nitrogen removal).

Materials and methods

Agro-industrial wastewater pretreatment and characterization

Five fresh wastewaters originated from different agro-industries in the region of Castilla y Leon (Spain) were used in this study: potato processing wastewater (PW), fish processing wastewater (FW), wastewater from an industry producing animal food (MW), lyophilized coffee manufacturing wastewater (CW) and wastewater from a yeast production factory previously subjected to anaerobic digestion (YW).

Samples were collected in 25-L polypropylene bottles and kept at 4 °C for 24 h prior to use. All agro-industrial wastewaters were pretreated by centrifugation at 15,317×*g* for 10 min at 23 °C and filtered through 0.40-μm filters. Therefore, only the soluble fraction of carbon, nitrogen and phosphorus was considered in the present study (Table 1).

Microorganisms and culture conditions

A mixed microalgae consortium, whose population structure in number of cells was composed of *Phormidium* (71 %), *Oocystis* (20 %) and *Microspora* (9 %), was collected from a HRAP treating diluted centrates at the Department of Chemical Engineering and Environmental Technology (University of Valladolid, Spain). Centrates constitute the liquid fraction derived from the centrifugation of the effluents from the anaerobic digestion of primary and secondary sludge in conventional activated sludge wastewater treatment plants. Due to their high $N\text{-}NH_4^+$ concentrations (>600 mg $N\text{-}NH_4^+ L^{-1}$), the dilution of these centrates with tap water was conducted prior to feeding the HRAP in order to avoid microalgae inhibition (González et al. 2008).

Photosynthetically oxygenated agro-industrial wastewater treatment

Unless otherwise specified, all tests described below were incubated at 30 °C (temperature controlled by a thermostatic water bath) under magnetic agitation (200 rpm) and diluted with tap water. Tests conducted with undiluted, 2, 4, 10, 20 and 100 times diluted pretreated agro-industrial wastewater will be herein referred as 1×, 2×, 4×, 10×, 20× and 100×, respectively. Control tests deprived of biological activity (200 mg L^{-1} of $CuCl_2$) will be referred as B× and were performed in order to assess any potential abiotic carbon, nitrogen or phosphorous degradation. All tests were cultivated in duplicate under a 12:12-h light/dark illumination regime at 76±4 μmol·photons $m^{-2} s^{-1}$.

Glass bottles of 1,250 mL (22 cm×10.5 cm height×diameter) were filled with 1,000 mL of undiluted, 2× and 4× diluted pretreated wastewater. The experimental series with CW was carried out at 2×, 10×, 20× and 100× dilutions due to the potential presence of toxic compounds for microalgae activity (Dinsdale et al. 1997). An additional test with 10× diluted wastewater was conducted with YW due to its high NH_4^+ concentration and therefore potential inhibition on microalgae (González et al. 2008). Control tests were carried out for each wastewater at 4× and at 20× in CW. All tests were inoculated with 2.2 mL of activated sludge and 22 mL of microalgae culture, resulting in final concentrations of 8± 1 mg volatile suspended solids L^{-1} (mg VSS L^{-1}) and 15±

Table 1 Composition of the soluble fraction of the five agro-industrial wastewaters evaluated

Parameters	Agro-industrial wastewaters				
	PW	FW	MW	CW	YW
TOC (mg L^{-1})	327	381	959	8,532	1,186
IC (mg L^{-1})	54	51	37	171	1,353
TN (mg N L^{-1})	69	82	197	766	703
N-NH_4^+ (mg L^{-1})	13	9	189	101	565
N-NO_2^- (mg L^{-1})	<D.L.	<D.L.	<D.L.	3	<D.L.
N-NO_3^- (mg L^{-1})	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.
$P_{soluble}$ (mg P L^{-1})	6	6	27	59	7
pH	7.1	7.7	6.0	7.0	8.1
C/N/P (g/g/g)	100/18/2	100/19/1	100/20/3	100/9/1	100/28/0.3

<*D.L.* below the HPLC-IC detection limit 1.1 mg N-NO_3^- L^{-1} and 1.5 mg N-NO_2^- L^{-1}

7 mg VSS L^{-1}, respectively. The bottles were finally flushed with helium, closed with butyl septa and then sealed with plastic caps in order to ensure that the biodegradation of the agro-industrial wastewaters proceeded exclusively driven by photosynthetic oxygenation.

Liquid samples were periodically withdrawn from the tests based on the time course of the total organic carbon (TOC) concentration and the maximum total sampling limitation of 15 % of the initial liquid volume. This conservative maximum sampling volume of 15 % was established in order to prevent experimental biases caused by the variations in the total liquid volume such as a vacuum-mediated air introduction to the bottles or changes with time in the agitation pattern and illuminated surface to volume ratio. Liquid samples were centrifuged for 10 min at 5,000 rpm (Kubota 5000, Japan) and filtered through 0.20-μm nylon filters to monitor the dissolved TOC, inorganic carbon (IC), total nitrogen (TN), N-NH_4^+, N-NO_2^-, N-NO_3^-, P-PO_4^{3-} and pH. Phosphorus concentration was measured at the beginning and end of the tests. Sampling during experimentation considered a maximum final volume withdrawal of 15 % of the initial value. Gas samples of 100 μL were also taken using gas-tight syringes (Hamilton Co., USA) to record CO_2, O_2 and N_2 concentrations in the flask's headspace by GC-TCD. Test monitoring stopped when the TOC concentration remained constant for at least two consecutive samplings. The final biomass in each test was collected by centrifugation (15,317 *x g* and 10 min) and dried at 105 °C for 24 h in order to determine its carbon and nitrogen (C and N) composition. Finally, it must be stressed that biomass concentration was not monitored in any of the biodegradation tests due to the formation of flocs in the algal culture (which hindered the accurate determination of biomass concentration by absorbance measurements) and the above mentioned sampling volume limitation during test monitoring. Thus, these preliminary biodegradability tests only considered the monitoring of the concentrations of the agro-industrial wastewater pollutants and biomass compositions.

Systematic determination of the limiting component in the biodegradation tests

In order to elucidate the limiting component (C, N or P) in the biodegradation of each wastewater, phosphorus (KH_2PO_4) at 10 mg P L^{-1} was added from a stock solution in one of the duplicate assays at the end of the experiment (when TOC concentration was constant for two samplings). If TOC concentration in this duplicate remained stable after KH_2PO_4 addition, ammonium (NH_4Cl) at 10 mg N L^{-1} was further added.

Analytical procedures

TOC, IC and TN concentrations were determined using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. Based on the different wastewater characteristics (turbidity and matrix effects), three different N-NH_4^+ determination methods were used: selective ammonia electrode Orion Dual Star (Thermo Scientific, The Netherlands) in PW, Nessler analytical method using a spectrophotometer U-2000 (Hitachi, Japan) at 425 nm in FW and MW, and distillation using a Bütcher distiller (KlejFlex K-360, Spain) in CW and YW. N-NO_3^-, N-NO_2^- and P-PO_4^{3-} were analysed via HPLC-IC using a Waters 515 HPLC pump coupled with a conductivity detector (Waters 432) and equipped with an IC-PAK Anion HC column (4.6×150 mm) and an IC-Pak Anion Guard-Pak (Waters). All analyses were carried out according to standard methods (Eaton et al. 2005). A Eutech CyberScan pH510 (Eutech Instruments, The Netherlands) was used for pH determination. The headspace concentrations of CO_2, O_2 and N_2 were analysed using a gas chromatograph (Varian CP-3800, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m×0.53 mm×15 μm) and CP-PoraBOND Q (25 m×0.53 mm×15 μm) columns. Injector and detector temperatures were maintained at 150 and 175 °C, respectively. Helium was used as the carrier gas at 13.7 mL min^{-1}. The light

intensity was measured with a LI-250A light meter (LI-COR Biosciences, Germany). The determination of the C and N biomass content was performed using a LECO CHNS-932 at the Instrumental Techniques Laboratory of Universidad Complutense de Madrid (Spain).

Results

TOC and TN concentrations in the pretreated wastewaters ranged, respectively, from 327 mg TOC L^{-1} and 69 mg TN L^{-1} in PW to 8,532 mg TOC L^{-1} and 766 mg TN L^{-1} in CW. The lowest IC concentration was recorded in FW (51 mg L^{-1}) and the highest (1,353 mg L^{-1}) in MW. Likewise, soluble phosphorus concentration varied from 6 mg P L^{-1} in PW and FW to 59 mg P L^{-1} in CW, while pH ranged between 6 (MW) and 8.1 (YW). In this context, the C/N/P (g/g/g) ratio varied from 100/9/1 in CW to 100/28/0.3 in YW (Table 1).

Biodegradability of agro-industrial wastewaters

The parameters monitored in the control tests remained constant regardless of the wastewater tested.

Biodegradation of potato processing wastewater

PW underwent fast carbon and nutrient removal regardless of the dilution applied. TOC removal occurred in 1×, 2× and 4× within the first 40 h of experimentation, with total removal efficiencies (REs) of 31±8, 38±3 and 54±8 % (Fig. 1a), respectively. Likewise, TN concentration decreased within the first 86 h, with TN REs of 19±1, 52±0.1 and 60±0.4 % in 1×, 2× and 4×, respectively. The decrease in TN in 1× and 2× was initially correlated with an increase in $N\text{-}NH_4^+$, while in 4×, $N\text{-}NH_4^+$ decreased from 3±0.1 to 1±0.1 mg L^{-1} (Fig. 1b, d). Neither NO_2^- nor NO_3^- were detected in the biodegradability assays regardless of the PW dilution. The O_2 concentration in the flask's headspace of 4× tests increased to 206±3 g m^{-3} in the first 134 h, concomitantly with a complete CO_2 depletion and a steady pH increase to 11.1±0.1 mediated by the decrease in IC concentration (data not shown). Similarly, complete CO_2 depletion in 2× occurred within the first 134 h, with progressive O_2 accumulation to 49±10 g m^{-3} and a pH increase 9.6±0.2 after 183 h. On the contrary, negligible O_2 headspace concentrations were recorded in 1×, where pH remained constant at 7.2±0.2 and the headspace CO_2 concentration increased from 40±2 to 100±11 g m^{-3} by the end of the experiment (Fig. 1c, e, f). Phosphorus REs of 89, 80 and 87 % were achieved in 1×, 2× and 4×, respectively. Finally, TOC concentration in 1× decreased from 200 to 160 mg L^{-1} as a result of $P\text{-}PO_4^{3-}$ addition , while no further decrease in

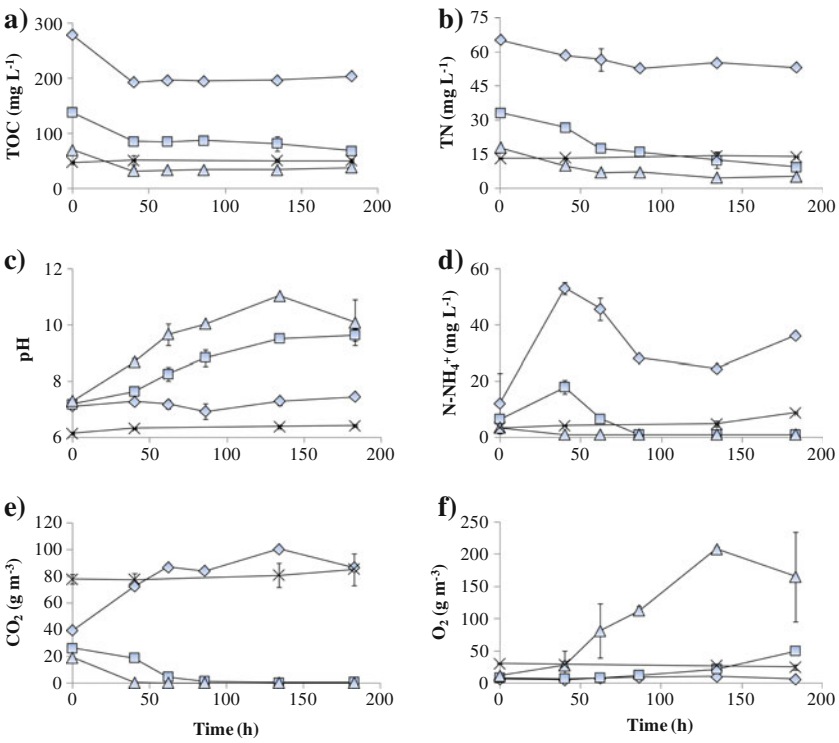

Fig. 1 Time course of (**a**) TOC, (**b**) TN, (**c**) pH, (**d**) $N\text{-}NH_4^+$, (**e**) CO_2 and (**f**) O_2 headspace concentrations during PW biodegradation in algal–bacterial photobioreactors in undiluted (—◇—), two times diluted (—□—), four times diluted (—△—) and control tests (—×—)

TOC concentration was recorded after $P\text{-}PO_4^{3-}$ and $N\text{-}NH_4^+$ addition in 2× and 4×.

Biodegradation of fish processing wastewater

FW underwent a progressive carbon and nutrient removal regardless of the dilution applied. The TOC concentration steadily decreased from 368±2 to 162±20 mg L^{-1} in 1× in 327 h, from 195±2 to 70±3 mg L^{-1} in 2× in 279 h, and from 75±1 to 45±2 mg L^{-1} in 4× in 156 h (Fig. 2a), which corresponded to TOC REs of 56±2, 64±2 and 40±1 %, respectively. Likewise, TN decreased concomitantly with TOC, resulting in TN REs of 64±1, 85±1 and 74±1 %, respectively (Fig. 2b). $N\text{-}NH_4^+$ concentration initially increased from 8±1 to 40±0.4 mg L^{-1} in 1× but decreased to 14±2 mg L^{-1} by the end of the test. A similar trend was recorded in 2× and 4× (Fig. 2d). Neither NO_2^- nor NO_3^- was detected in the cultivation broth regardless of the dilution tested. The headspace CO_2 concentration initially increased during the first hours of experimentation up to 142±2, 63±3 and 22±8 g m^{-3} in 1×, 2× and 4×, respectively, followed by a faster decrease until complete depletion (Fig. 2e). The recorded pH values increased concomitantly with the decrease in CO_2 concentration to maximum values of 8.1±0.1, 8.7±0.1, and 9.3 ±1.4 in 1×, 2× and 4× tests, respectively (Fig. 2c). The headspace oxygen concentration remained close to zero in 1× and 2× during the entire test and gradually increased from 13±1 to 44±1 g m^{-3} in 4× tests (Fig. 2f). Due to the low phosphorus concentration (≤6 mg P L^{-1}) and the coloured nature of FW, the final $P\text{-}PO_4^{3-}$ removals were not experimentally determined. No further decrease in TOC and TN concentrations (which remained >5 mg N L^{-1}) was recorded after $P\text{-}PO_4^{3-}$ addition.

Biodegradation of animal feed production wastewater

The biodegradation of MW diluted 2× and 4× in enclosed algal–bacterial systems was characterized by an initial lag phase followed by a rapid decrease in carbon concentrations, which resulted in final TOC REs of 49±1 % in 374 h and 42±2 % in 246 h, respectively (Fig. 3a). TN and $N\text{-}NH_4^+$ concentrations were similar and correlated throughout the entire biodegradation process (Fig. 3b, d). The final TN REs in 2× and 4× were 62±2 and 80±2 %, respectively. Neither NO_2^- nor NO_3^- was detected in the biodegradability assays regardless of the MW dilution. The initial pH values steadily increased from pH 6.7 to 9.6 and 10.4 in 2× and 4×, respectively (Fig. 3c), while the initial CO_2 present in the flask headspace was completely removed by the end of the test in 2× and 4× (Fig. 3e). On the other hand, the O_2 concentration in the flask headspace rapidly decreased during the first 22 h in 2× and 4×, while a sudden O_2 concentration increase to 120± 5 g m^{-3} was recorded in 4× after 175 h (Fig. 3f). Phosphorus removals in 2× and 4× achieved final values of 83 ±5 and 57±9 %, respectively. No significant biological

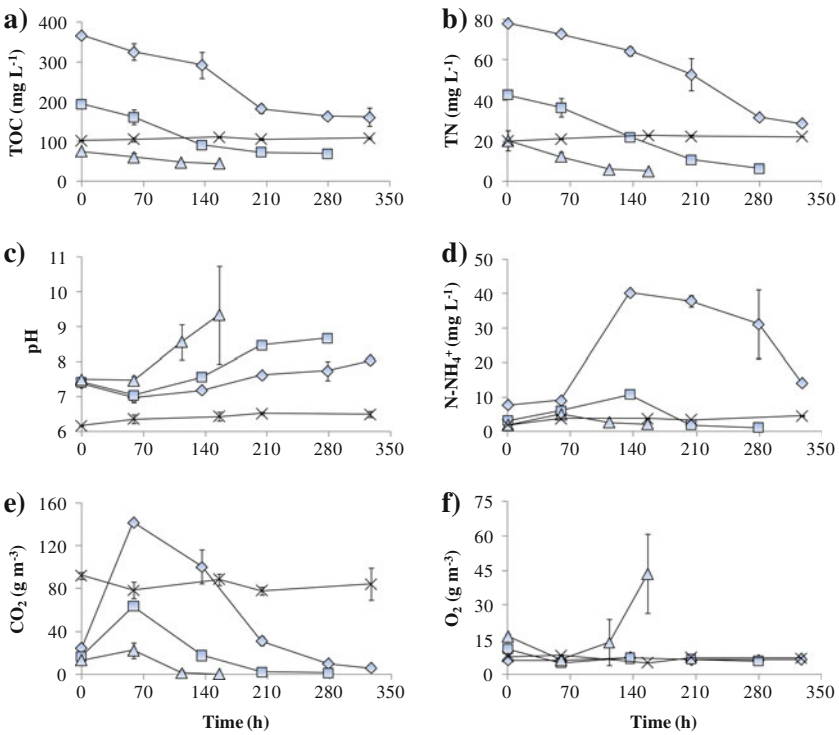

Fig. 2 Time course of (**a**) TOC, (**b**) TN, (**c**) pH, (**d**) $N\text{-}NH_4^+$, (**e**) CO_2 and (**f**) O_2 headspace concentrations during FW biodegradation in algal–bacterial photobioreactors in undiluted (—◇—), two times diluted (—□—), four times diluted (—△—) and control tests (—×—)

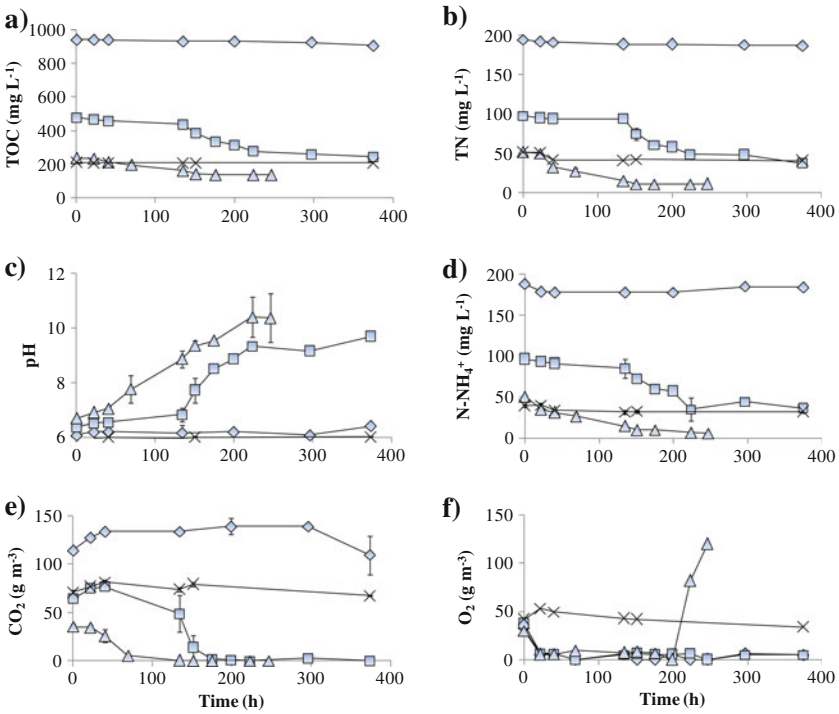

Fig. 3 Time course of (**a**) TOC, (**b**) TN, (**c**) pH, (**d**) $N\text{-}NH_4^+$, (**e**) CO_2 and (**f**) O_2 headspace concentrations during MW biodegradation in algal–bacterial photobioreactors in undiluted (—◇—), two times diluted (—□—), four times diluted (—△—) and control tests (—×—)

activity, estimated from the variation in TOC, TN, pH and CO_2 concentrations, was recorded in undiluted tests despite the recorded O_2 depletion within the first 22 h. No further decrease in TOC was recorded after $P\text{-}PO_4^{3-}$ addition in none of the assays, while $N\text{-}NH_4^+$ was not supplemented based on its high concentrations at the end of the tests.

Biodegradation of lyophilized coffee manufacturing wastewater

Organic matter biodegradation in CW tests was characterized by a rapid initial TOC decrease, which resulted in final TOC REs of 18±2, 23±4, 21±8 and 56±2 % in 2×, 10×, 20× and 100×, respectively, (Fig. 4a). TN decreased concomitantly with TOC, with final REs of 8±2, 27±8, 32±13 and 80±4 % in 2×, 10×, 20× and 100×, respectively. The time course of $N\text{-}NH_4^+$ was characterized by an initial concentration decrease during the first stages of the biodegradation process, followed by a slight increase (Fig. 4d). Similar to the observations in the previous agro-industrial wastewaters tested, neither NO_2^- nor NO_3^- was recorded in the cultivation broths. The CO_2 headspace concentration increased to 1,542±107, 408±1 and 232±4 g m^{-3} in 2×, 10× and 20×, respectively, while pH decreased respectively to 4.9±0.2, 5.2±0.0 and 6.1±0.2 by the end of the test. The headspace O_2 concentration in these assays was depleted after 95 h. On the contrary, CO_2 concentration in 100× CW was negligible while pH and O_2 concentrations increased to 9.6±0.4 and 185±49 g m^{-3}, respectively, by the end of the process (Fig. 4c, e, f). The spectrophotometric determination of phosphorus concentration was not possible due to the coloured nature of CW and the relatively high HPLC-UV detection limit (1.6 mg $P\text{-}PO_4^{3-}L^{-1}$), which only allowed the determination of phosphorus concentration in 2× ($P\text{-}PO_4^{3-}$ RE of 8±1 %). At the end of the assay, the addition of $P\text{-}PO_4^{3-}$ at 10 mg L^{-1} in one of the duplicates did not result in significant variations in TOC or TN concentration. $N\text{-}NH_4^+$ was not supplemented based on its high concentrations at the end of the tests.

Biodegradation of yeast production wastewater

Low TOC REs of 27±10 and 33±2 % were achieved in 4× and 10× YW, respectively (Fig. 5a), in 395 h. The TN REs achieved in 4× and 10× accounted for 12±1 and 50±1 %, respectively, while $N\text{-}NH_4^+$ REs were 23±2 and 38±1 %, respectively. Neither NO_2^- nor NO_3^- was recorded during the experimental period, while pH increased from pH 8 to 8.6 in 4× and from pH 8.2 to 9.1 in 10×. This increase was correlated to a headspace CO_2 uptake from 27±0.2 to 15±5 g m^{-3} and from 13±0.1 to 1±0.1 g m^{-3} in 4× and 10×, respectively, concomitantly with an O_2 increase from 9±0.1 to 153±10 g m^{-3} and from 19±2 to 406±3 g m^{-3}, respectively. Once again, the spectrophotometric determination of phosphorus concentration was not possible due to the coloured nature of the YW. The

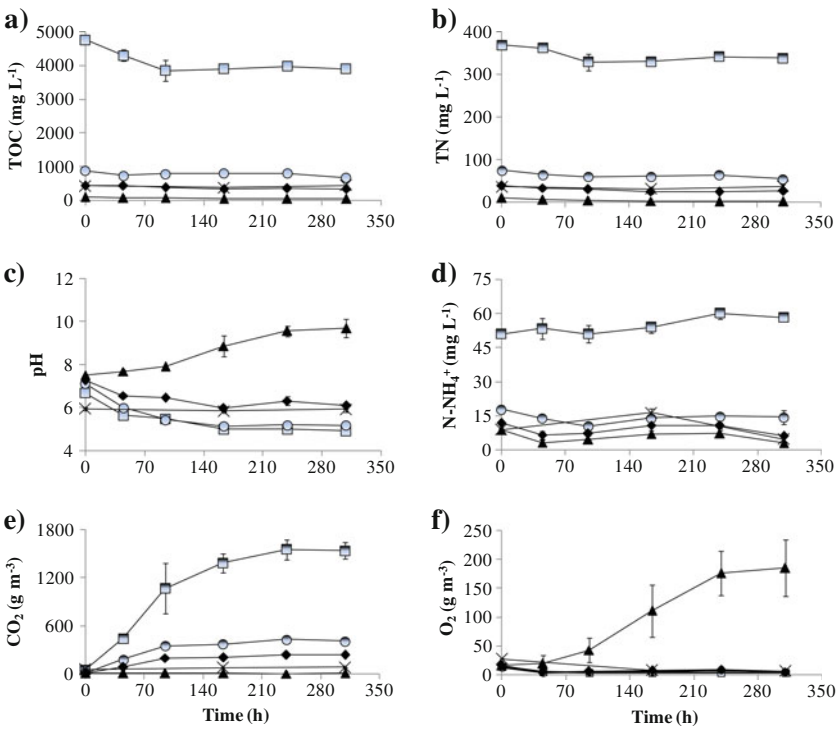

Fig. 4 Time course of (**a**) TOC, (**b**) TN, (**c**) pH, (**d**) N-NH_4^+, (**e**) CO_2 and (**f**) O_2 headspace during CW biodegradation in algal–bacterial photobioreactors in 2 times diluted (—□—), 10 times diluted (—○—), 20 times diluted (—◆—), 100 times diluted (—▲—) and control tests (—✕—)

addition of P-PO_4^{3-} to one of the duplicates at the end of the test resulted in a TOC concentration decrease from 226 to 186 mg L^{-1} in 4× and from 93 to 48 mg L^{-1} in 10×. No significant variations in TOC, TN and headspace O_2 concentrations were recorded in 1× and 2× tests, which were characterized by high N-NH_4^+ concentrations (565±14 and 271±1 mg L^{-1} in 1× and 2×, respectively) (Fig. 5a, b, f). The pH remained approximately constant at

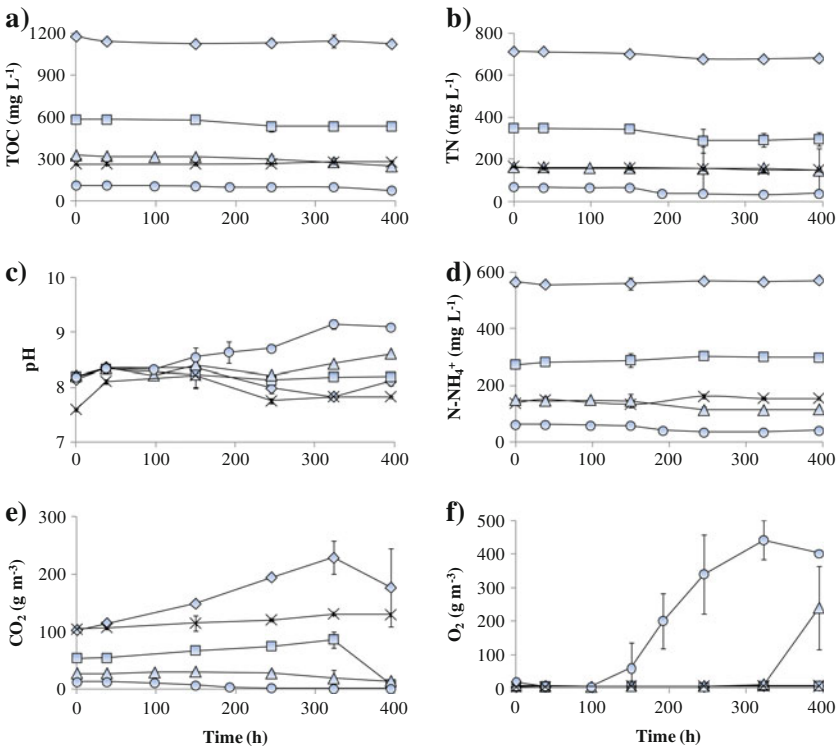

Fig. 5 Time course of (**a**) TOC, (**b**) TN, (**c**) pH, (**d**) N-NH_4^+, (**e**) CO_2 and (**f**) O_2 headspace concentrations during YW biodegradation in algal–bacterial photobioreactors in undiluted (—◇—), 2 times diluted (—□—), 4 times diluted (—△—), 10 times diluted (—○—) and control tests (—✕—)

Table 2 Carbon and nitrogen contents in percentage of the harvested biomass in the respective wastewaters and dilutions at the end of the biodegradability tests

Dilutions	Agro-industrial wastewaters									
	PW		FW		MW		CW		YW	
	C	N	C	N	C	N	C	N	C	N
1×	51.0	8.8	56.8	8.4	N.B.O.	N.B.O.	N.T.	N.T.	N.B.O.	N.B.O.
2×	44.3	7.1	53.8	7.5	46.2	9.0	50.6	9.5	N.B.O.	N.B.O.
4×	42.8	6.5	42.1	5.7	45.2	8.6	N.T.	N.T.	38.9	7.1
10×	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	49.6	8.7	35.1	6.2
20×	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	49.0	8.4	N.T.	N.T.
100×	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	40.4	4.1	N.T.	N.T.

Due to the low amount of biomass formed in some biodegradation tests, the experimental determination of the P content in the biomass was not performed

Carbon and nitrogen biomass content was analysed in the duplicate batch bottles in which the determination of the limiting component was not carried out

N.B.O. non biodegradation observed, *N.T.* non-tested

pH 8.1 and 8.2 in 1× and 2×, respectively (Fig. 5c). The headspace CO_2 concentration increased slightly in 1× and 2× from 102±2 to 229±29 g m^{-3} and from 54±3 to 86±14 g m^{-3}, respectively, with a sudden decrease at the end of the process (Fig. 5e).

Carbon and nitrogen biomass content

A higher carbon and nitrogen content in the biomass was recorded when decreasing wastewater dilution regardless of the treated wastewater (Table 2). Despite its high inorganic carbon concentration, the lowest carbon content was recorded in the biomass harvested from YW biodegradation in 4× and 10× tests (39 and 35 %, respectively).

Discussion

The preliminary analysis of the agro-industrial wastewaters tested showed a wide range of concentrations for the different parameters evaluated (Table 1). Likewise, the biodegradability tests showed varied carbon and nutrient removals depending on the agro-industrial wastewater evaluated and the dilution tested. The optimal C/N/P ratio in a wastewater for microalgae growth reported in literature is 100/18/2 (Oswald 1988), which corresponded to the C/N/P ratio of PW (Table 1) as well as to the fastest carbon and nitrogen removal rates recorded among the five wastewaters. The C/N/P ratio of FW and MW was also similar to this optimal ratio and supported efficient carbon and nutrient removals. CW had a low C/N ratio, while YW had both a low P content and C/N ratio, which could explain their low biodegradability. These results are in agreement with those recently reported by Yan et al. (2013), who recorded a significant influence of the C/N ratio of domestic sewage on the microalgae-based removal of nitrogen and phosphorus, with an optimum value of 5:1. Likewise, Cai et al. (2013) observed different N and P removals depending on their initial ratio in the cultivation medium.

The fact that negligible C, N and P removals were recorded in the control tests confirmed that both carbon and nutrient removals in the biotic tests occurred exclusively supported by the symbiosis between microalgae and bacteria. In this context, TOC removal occurred via heterotrophic bacterial oxidation, which entailed a significant O_2 consumption and CO_2 production. On the other hand, based on the absence of NH_4^+ nitrification and the enclosed nature of the test, IC was removed photosynthetically via microalgae assimilation, with the subsequent increase in the pH of the cultivation medium and the release of O_2 as a result of photosynthetic CO_2 consumption (González et al. 2008). At this point, it should be also stressed that despite low O_2 concentrations were recorded throughout most biodegradation stages, TOC removal occurred aerobically due to the continuous in situ oxygen supply by microalgae, as noted earlier by Guieysse et al. (2002). NH_3 stripping to the headspace could have contributed to TN removal based on the high pH levels recorded at the end of microalgal cultivations. However, the low equilibrium headspace NH_3 concentrations estimated theoretically suggested that nitrogen assimilation into biomass was the main N removal mechanism.

In this particular study, floc formation in the microalgal–bacterial cultures hindered the accurate determination of biomass concentration by culture absorbance measurements, which is an analytical technique with low sampling volume requirements. Biomass quantification through conventional total suspended solid analyses was not carried out due to the

high sampling volume needed for this analytical technique (≥25 mL due to the low biomass concentrations in the systems, visual observation), which would have entailed the withdrawal of total sampling volumes far above the conservative maximum final sampling volume of 15 % selected for these standard biodegradability tests. However, despite the fact that biomass concentration was not periodically monitored, the impact of a biomass-mediated light scattering or light limitation on the results obtained was negligible due to the low biomass concentration supported by the final carbon removal recorded. Thus, the maximum final biomass concentration estimated based on the total carbon removed and the C content of the final harvested biomass was 0.17 g L^{-1} in 2× FW tests, which likely supported an efficient light distribution in the bottles. Finally, the fact that the soluble fraction of the agro-industrial wastewaters was used in the biodegradability assays did not invalidate the results here obtained due to the fact that any particulate organic matter present in the wastewaters ultimately would be hydrolyzed by the microorganisms prior to cell uptake in soluble form. Full-scale wastewater treatment plants are nowadays operated with preliminary screening units, primary settlers and rotary fine screens, which generate wastewaters with a low content of particulate pollution (similar to those obtained here by filtration) (Metcalf 2003). Besides, the contribution of the particulate organic matter to the total C, N and P load in the raw wastewaters evaluated in this study was low.

Biodegradability of agro-industrial wastewaters

The biodegradable total organic carbon was the limiting component during wastewater treatment in most of the evaluated agro-industrial effluents and dilutions. The high pH values and the high N-NH_4^+ concentrations in some wastewaters might have also inhibited the biodegradation process (Craggs et al. 2011). Thus, the results here obtained highlight the need for an external carbon source (CO_2) supply, the implementation of pH control strategies and the dilution of the high N-NH_4^+ concentrations in conventional HRAPs for optimal wastewater treatment (Craggs et al. 2011; De la Noüe et al. 1994).

Biodegradation of potato processing wastewater

Carbon and nutrient concentrations in potato processing wastewater were relatively low compared to the other agro-industrial wastewaters tested despite its neutral pH and optimal C/N/P ratio (Table 1). The rapid TOC removal and increase in the cultivation pH in 2× and 4× suggest the occurrence of an active microalgae population capable of satisfying the bacterial oxygen demand of the biodegradation process (Olguín 2003). The TN decrease correlated with an increase in N-NH_4^+ concentration for 1× and 2×, which was likely due to the ammonification of the dissolved organic N (which constitutes the major fraction of TN in PW) (Fig. 1b, d). However, N-NH_4^+ concentration decreased accordingly to TN in 4× probably mediated by the low ammonification rate as a consequence of the low organic N concentration. The absence of NO_2^- and NO_3^- in the biodegradation assays, together with the enclosed nature of the tests, suggests that most of the ammonium was removed via assimilation into biomass prior to ammonification. The absence of nitrification in this series of tests was probably mediated by the low fraction of nitrifying bacteria present in the inoculum (15± 7 mg VSS L^{-1} of total bacterial biomass), their low growth rate and the high initial oxygen demand of the TOC-degrading heterotrophic bacteria (which outcompete nitrifiers). Likewise, the high pHs might have inhibited NH_4^+ nitrification despite the high O_2 concentrations at the end of the tests in 2× and 4× (Metcalf 2003). Although TOC removal stopped after 50 h in all dilution tests, the fact that O_2 concentration only increased significantly in 4× (due to its higher microalgae activity and the lower oxygen demand of the wastewater) and in a lower extent in 2×, suggests a partial inhibition of microalgae activity mediated by the wastewater. The systematic determination of the limiting component in this biodegradation test series concluded that the limiting component in 1× was phosphorus, while biodegradable TOC might limit the biodegradation process in 2× and 4×. However, a potential inhibition mediated by the high pH values recorded in 2× and 4× was more likely to inhibit bacterial activity than the absence of biodegradable TOC.

Biodegradation of fish processing wastewater

Fish processing wastewater showed similar characteristics than PW, although its pH was slightly higher (Table 1). The progressive TOC decrease and the increase of CO_2 concentrations during the first hours of experimentation indicated an intense bacterial activity. However, the decrease in CO_2 concentration concomitant with the increase in pH and O_2 concentrations indicated a strong microalgae activity by the end of the tests. The high photosynthetic activity and the low oxygen demand during the last stages of the FW biodegradation process resulted in the high O_2 concentrations recorded in 4×. Ammonification was identified as the main responsible of the initial N-NH_4^+ concentration increase in the assays. The difference between TN and N-NH_4^+ concentrations and the absence of NO_2^- and NO_3^- clearly indicate that organic nitrogen was the main component of the total nitrogen in FW. The reasons underlying the lack of nitrification in this test were likely similar to those discussed in PW, and this rationale could be applied to all agro-industrial wastewaters tested. The biodegradable TOC was the limiting component of the process regardless of the dilution.

Biodegradation of animal feed production wastewater

The animal feed production wastewater was initially characterized by moderate carbon and nutrient concentrations compared to the other agro-industrial wastewaters tested, a favourable C/N/P ratio and a slightly acidic pH (Table 1). The lag phase recorded for TOC and TN removal in 2× and 4×, together with the rapid oxygen depletion, suggests that microalgae activity likely limited the biodegradation process during the initial stages of the processes. On the other hand, the O_2 concentration increase recorded in 4× at the end of the test was likely due to the depletion of all potential oxidizable substrates (TOC) and to active microalgal photosynthesis. In any case, the absence of O_2 in the bottle's headspace during most of the biodegradation assays likely inhibited NH_4^+ nitrification. In this context, the high correlation between TN and N-NH_4^+ concentrations indicated that N-NH_4^+ was the only contributor to TN. The increase in pH in 2× and 4× was probably mediated by an intense photosynthetic activity (CO_2 uptake) by the end of the test and a potential release of basic metabolites. The concentration of biodegradable TOC likely limited the biodegradation process in this particular wastewater based on the high N-NH_4^+ concentrations and absence of biological activity after PO_4^{3-} addition. In the particular case of 1×, the high N-NH_4^+ concentrations or the presence of inhibitory compounds might have exerted a detrimental effect on microalgae activity and therefore on the O_2 supply.

Biodegradation of lyophilized coffee manufacturing wastewater

The TOC and nutrient concentrations in the lyophilized coffee manufacturing wastewater ranked the highest among the five agro-industrial wastewaters tested. A neutral pH and a low C/N ratio, compared to the optimum reported, also characterized CW (Table 1). The low TOC REs recorded highlighted the highly recalcitrant nature of this wastewater. The initial N-NH_4^+ concentration decrease was likely due to assimilation into algal-biomass (based on the absence of nitrifying activity and enclosed nature of the tests), while the subsequent increase was likely due to ammonification of N-organic. The differences in TN and N-NH_4^+ concentrations, along with the absence of NO_2^- and NO_3^-, suggest that organic N was the main nitrogenous component in CW. The highest bacterial activity was recorded in 2× as shown by the highest CO_2 concentrations and the lowest pHs among the dilutions tested. On the other hand, the highest microalgae activity was recorded in 100× based on the highest O_2 concentrations and pHs. The high CO_2 concentrations recorded in the absence of both O_2 and microalgae growth in 2×, 10× and 20× (visual observation) suggest the occurrence of anaerobic organic matter biodegradation, which could also explain the recorded decrease in the pH of the cultivation broths. The biodegradable TOC was the limiting component of the biodegradation process regardless of the wastewater dilution. However, a potential inhibition of the algal–bacterial community by some toxic compounds present in the wastewater cannot be ruled out based on the high microalgae activity at the highest CW dilution.

Biodegradation of yeast production wastewater

Yeast production wastewater was characterized by a low soluble phosphorus, moderate TOC and high N-NH_4^+ and IC concentrations compared to the rest of agro-industrial wastewaters evaluated (Table 1). The low TOC removal rates together with the gradual CO_2 accumulation within the first hours of experimentation suggested an initial low bacterial activity in 4× and 10×. However, photosynthesis was more likely to limit the biodegradation process based on the low O_2 concentrations recorded in the headspace. In the absence of NO_3^- and NO_2^-, the results suggest that N-NH_4^+ and N-organic were the main contributors to TN (94.8 and 5.2 %, respectively). The high buffer capacity of this wastewater, as a result of its high IC concentrations (Table 1), limited the pH increase in tests 4× and 10×. The systematic determination of the limiting component in 4× and 10× confirmed that phosphorus was the limiting substrate in the biodegradation of this anaerobically pretreated wastewater. On the other hand, the high N-NH_4^+ concentrations of this wastewater likely inhibited microalgae activity in 1× and 2× and therefore the biodegradation process. However, the fact that the variations in CO_2 concentration were not correlated to a decrease in the TOC, TN or O_2 concentrations suggests that they might have been just abiotically induced by variations in pH (Fig. 5c).

Carbon and nitrogen biomass content

The increase in the cultivation medium of the C and N available for biomass growth when decreasing wastewater dilution likely induced the increase in carbon and nitrogen contents recorded in the biomass harvested at the end of the biodegradation tests. The C and N contents were within conventional reported ranges (C 40–60 %; N 4–9 %) (Grobelaar 2004; Cabanelas et al. 2013). This study confirmed that C and N recovery during microalgae-based wastewater treatment constitutes an opportunity to enhance the economic viability and sustainability of agro-industry.

In conclusion, in the absence of inhibitory compounds, the initial C/N/P ratio of the agro-industrial wastewater was correlated with its biodegradability. PW and FW were effectively treated in terms of carbon and nutrient removal. Low carbon and nutrient removal was recorded in CW and YW, the most recalcitrant wastewaters. Despite the low TOC removal efficiencies recorded, the biodegradable organic carbon was in

most cases the limiting component in the biodegradation, although eventually the combination of high pH values and high N-NH_4^+ concentrations might have also limited wastewater treatment. A similar carbon and nitrogen biomass content, higher than the lower dilution applied, was recorded regardless of the target wastewater.

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Carbon and nutrient removal from centrates and domestic wastewater using algal-bacterial biofilm bioreactors

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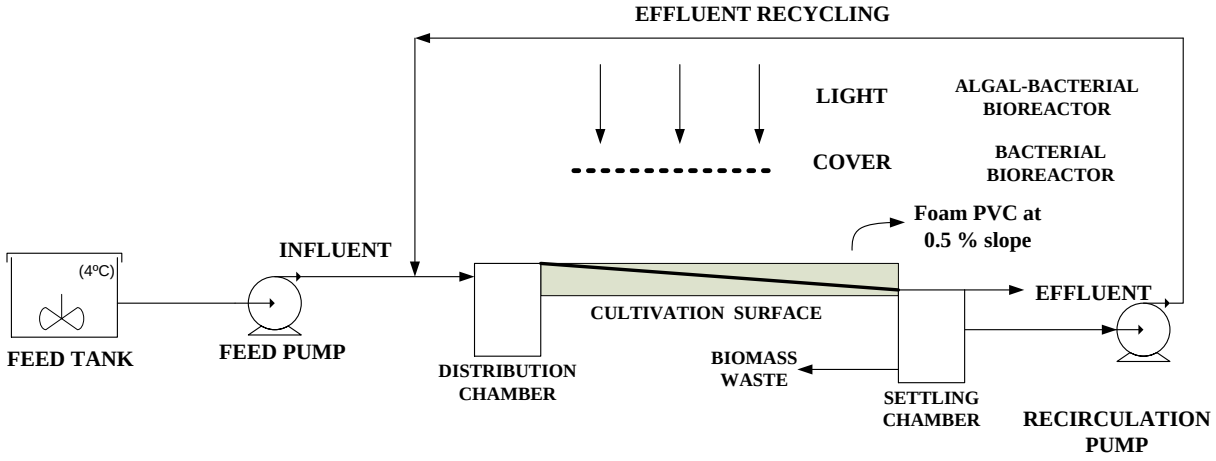
Chapter 4



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Carbon and nutrient removal from centrates and domestic wastewater using algal–bacterial biofilm bioreactors

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HIGHLIGHTS

- Comparable carbon removal in the algal–bacterial and bacterial biofilm bioreactors.
- Twice higher nutrient removals in the algal–bacterial biofilm bioreactor.
- Similar rate of assimilation in algal biomass and stripping to C and N removal.
- Phosphorus removed by assimilation into algal–bacterial biomass.
- A high water footprint recorded in the algal–bacterial biofilm reactor.

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ABSTRACT

The mechanisms of carbon and nutrient removal in an open algal–bacterial biofilm reactor and an open bacterial biofilm reactor were comparatively evaluated during the treatment of centrates and domestic wastewater. Comparable carbon removals (>80%) were recorded in both bioreactors, despite the algal–bacterial biofilm supported twice higher nutrient removals than the bacterial biofilm. The main carbon and nitrogen removal mechanisms in the algal–bacterial photobioreactor were assimilation into algal biomass and stripping, while stripping accounted for most carbon and nitrogen removal in the bacterial biofilm. Phosphorus was removed by assimilation into algal–bacterial biomass while no effective phosphorous removal was observed in the bacterial biofilm. Carbon, nitrogen and phosphorus removals of 91 ± 3%, 70 ± 8% and 85 ± 9%, respectively, were recorded in the algal–bacterial bioreactor at 10 d of hydraulic retention time when treating domestic wastewater. However, the high water footprint recorded (0.5–6.7 L m^{-2} d^{-1}) could eventually compromise the environmental sustainability of this microalgae-based technology.

1. Introduction

The rapid growth of human population over the past century from 1600 to 6700 million people (UN, 2011) has entailed the generation of huge amounts of domestic wastewaters worldwide (Craggs et al., 1996a). Despite their high geographic and seasonal variability in terms of flowrates and contaminant concentrations, domestic wastewaters are often characterized by their high organic matter and nutrient loads (Metcalf and Eddy, 2003), whose uncontrolled disposal into the environment is gradually deteriorating water quality in lakes and rivers as a result of eutrophication and O_2 depletion (de Bashan and Bashan, 2010). In this context, the increasing public concern about preservation of natural water resources has triggered the development of a wide range wastewater treatment technologies and the enforcement of stricter wastewater discharge regulations (de la Noüe et al., 1992). However, conventional wastewater treatment technologies such as aerobic activated sludge processes or anaerobic digestion still present severe technical–economic limitations caused by their high energy requirements and poor nutrient removals, respectively (de Godos et al., 2010).

In this regard, algal–bacterial processes constitute an environmentally friendly alternative for the treatment of domestic wastewaters able to overcome the above mentioned limitations (Muñoz and Guieysse, 2006). These sun-powered, operationally simple processes are based on the symbiotic interactions between microalgae and bacteria. In brief, the oxygen photosynthetically produced by microalgae in the presence of light and CO_2 is used by bacteria to *in situ* oxidize the organic matter and nutrients present in the wastewater, producing in return the CO_2 needed for micro-

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algae photosynthesis (Muñoz and Guieysse, 2006). The first applications of this technology were implemented in the mid 1950s in California for the treatment of domestic wastewaters in the so called high-rate-algal-ponds (HRAPs) (Oswald, 1988). Although successful nutrient and organic matter removals have been consistently recorded in HRAPs (de Godos et al., 2009a), the harvesting of the microalgae produced still constitutes one of the operational limitations of this technology due to their small cell size, strong negative surface charge, low and varying microalgae concentrations, and similar density of microalgae cells to water (Park et al., 2011). For instance, García et al. (2000) reported maximum microalgal biomass removal efficiencies of 80% in a settler operated at surface loading rates of 2 $m^3 m^{-2} d^{-1}$, which are 20 times lower than those typically applied in activated sludge settlers.

Biofilm photobioreactors represent an innovative approach to enhance biomass harvesting in microalgae-based wastewater treatment processes (Boelee et al., 2011). Hence, biofilm photobioreactors allow for the simultaneous production of a biomass-free effluent and an easily harvestable biomass (de Godos et al., 2009b). However, the high cost of microalgae immobilization materials (e.g. carrageenan, chitosan and alginate), together with their structural weakness during long term operation (especially in the presence of high PO_4^{-3} concentrations), have promoted the development of a new generation of enclosed biofilm photobioreactors. These innovative phototrophic biofilm bioreactors are based on the sole biofilm attachment to the photobioreactor walls and have shown promising carbon and nutrient removal capacities during the treatment of secondary domestic wastewaters (Boelee et al., 2011; Guzzon et al., 2008; Zamalloa et al., 2013) and both digested and raw manure effluents (Craggs et al., 1996b; Kebede-Westhead et al., 2004; Muñoz et al., 2009; Pizarro et al., 2002; Wilkie and Mulbry, 2002). For example, uptake rates of nitrate and phosphate from municipal secondary effluents of up to 1.0 g NO_3^- $m^{-2} d^{-1}$ and 0.34 g P $m^{-2} d^{-1}$, respectively, have been reported in an open microalgal biofilm photobioreactor (Boelee et al., 2011). Likewise, up to 83 ± 25% and 91 ± 12% of the total nitrogen and phosphorus removed from dairy manure wastewater, respectively, were recovered in the harvested microalgal biomass in an algal turf scrubber (ATS) (Mulbry et al., 2008). Due to the fact that the previous studies of algal–bacterial biofilm bioreactors were based on the treatment of wastewaters previously treated aerobically or anaerobically (where most of the carbon content was removed), these studies (Table 1) only focused on nitrogen and phosphorus removal. However, despite the promising performance of algal biofilm bioreactors for the treatment of secondary domestic and anaerobically digested livestock effluents, there is no single study focused on the evaluation of the potential of these innovative biofilm photobioreactors as a core technology for the treatment of centrate and raw domestic wastewaters.

This research was based on the previously reported successful nutrient removal performance of algal–bacterial biofilm bioreactors treating diluted manure effluents and on their potential for algal biomass retention in order to reduce the harvesting costs, under the hypothesis that the characteristics of these photobioreactor configurations and the mechanisms underlying pollutant removal in algal–bacterial biofilms can support an efficient C, N, P and solid removal during the treatment of centrates and domestic wastewater. Thus, in this work, the potential of an open algal–bacterial biofilm photobioreactor and a control open bacterial biofilm bioreactor to remove carbon, nitrogen and phosphorous from diluted centrates and domestic wastewaters was comparatively evaluated for 220 days. The influence of the hydraulic residence time and effluent recycling flowrate on wastewater treatment performance was investigated. Finally, the influence of carbon and nutrient loading rate on biomass composition was also assessed.

2. Methods

2.1. Microorganisms and culture conditions

The biofilm bioreactors were inoculated with a microalgal consortium collected from a pilot HRAP treating diluted centrates at the Department of Chemical Engineering and Environmental Technology (University of Valladolid, Spain), and with activated sludge from Valladolid wastewater treatment plant (WWTP).

2.2. Wastewaters

Centrate wastewater was obtained by centrifugation of the anaerobically digested mixed sludge of Valladolid WWTP (Spain). Centrates were diluted with tap water due to the potential inhibition of microalgae growth by their high NH_4^+ concentrations (González et al., 2008). Raw domestic wastewater was directly collected from a public sewer nearby the Department of Chemical Engineering and Environmental Technology of Valladolid University. The domestic wastewater was pre-treated by screening and primary sedimentation (3 h of retention time) (Table 2).

2.3. Experimental set-up

The experimental set-up consisted of two identical 31 L biofilm bioreactors with a cultivation surface of 0.5 m^2 (1 m long and 0.5 m wide) at a slope of 0.5% in order to maintain a uniform downflow (with a maximum water layer of 0.5 cm) and two chambers of

Table 1
Operational parameters and nutrient removal performance in algal–bacterial biofilm photobioreactors treating different types of wastewaters.

Photobioreactor	Wastewater	TN load ($g m^{-2} d^{-1}$)	TN removed (%)	TP load ($g m^{-2} d^{-1}$)	TP removed (%)	Reference
ATS + ultraviolet system (UV)	Secondary sewage	8–0.8	50 (n.l.r.)	1.4–3	52 (l.l.r.)–40 (h.l.r.)	Craggs et al. (1996b)
BAGC	Dairy manure	1.03–0.64	42 (l.l.r.)–38 (h.l.r.) (a.b.)	0.16–0.08	100 (l.l.r.)–69 (h.l.r.) (a.b.)	Wilkie and Mulbry (2002)
Laboratory-scale ATS	Dairy manure	0.80–0.05	84 (l.l.r.)–73 (h.l.r.)	0.16–0.01	100 (l.l.r.)–98 (h.l.r.)	Pizarro et al. (2002)
Laboratory-scale ATS	Anaerobically digested dairy manure effluent	3.7–0.8	35.9 ± 11.4 (a.b.)	0.58–0.12	34.3 ± 6.2 (a.b.)	Kebede-Westhead et al. (2004)
Outdoors ATS raceways (Maryland, USA)	Anaerobically digested dairy manure effluent	2.5–0.3	83 ± 25 (l.l.r.)–57 ± 13 (h.l.r.) (a.b.)	0.40–0.05	91 ± 12 (l.l.r.)–62 ± 11 (h.l.r.) (a.b.)	Mulbry et al. (2008)
Flow cell (algal biofilm)	Effluent from a municipal WWTP	4.53–0.11	22	0.50–0.01	26	Boelee et al. (2011)

a.b. = recovery in algal biomass.
l.l.r. = low loading rate; h.l.r. = high loading rate; n.l.r. = no loading rate influence.

Table 2
Composition of the centrate and pre-treated domestic wastewater.

Wastewater	TOC (mg L^{-1})	IC (mg L^{-1})	TN (mg L^{-1})	NH_4^+ (mg N L^{-1})	PO_4^{3-} (mg P^- L^{-1})	C:N:P ratio	TSS (g L^{-1})	pH
Raw centrate	76 ± 7	717 ± 36	666 ± 36	646 ± 29	101 ± 31	100:84:13	0.01 ± 0.01	7.47 ± 0.02
Domestic wastewater	181 ± 69	100 ± 23	91 ± 14	66 ± 16	7 ± 3	100:32:02	0.35 ± 0.10	7.11 ± 0.35

Neither NO_2^- nor NO_3^- were detected in the raw wastewaters.

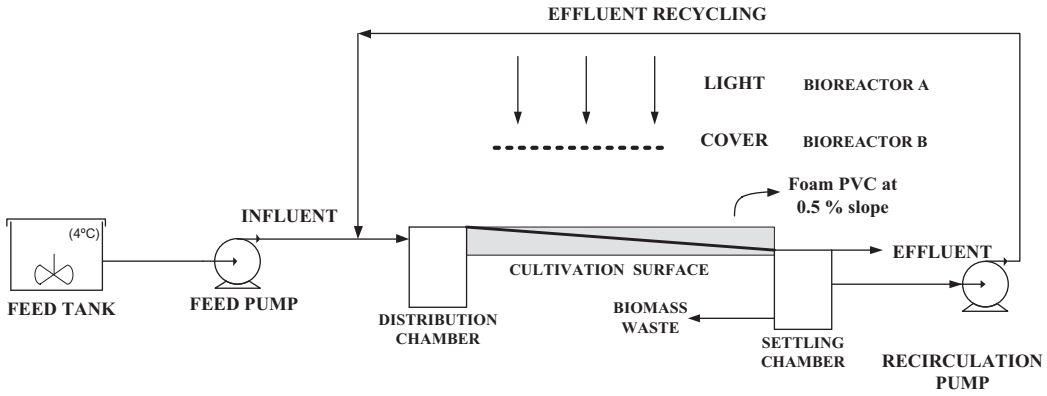

Fig. 1. Schematic diagram of the laboratory scale biofilm bioreactors.

15.5 L as distribution and settling units, respectively (Craggs et al., 1996b). Both the raw wastewater and effluent recirculation entrances were located in the distribution chamber. The mixture of wastewater and recycled effluent was distributed homogeneously from the distribution chamber along the cultivation surface and collected in the settling chamber, where both the effluent and recirculation outlets were located (Fig. 1).

The bioreactors were built in 5 mm thick foam PVC. The cultivation surfaces were sanded in order to promote microbial attachment (Boelee et al., 2011) (Fig. 1). The biofilm bioreactor A was exposed to a light:dark regime of 16:8 h:h at an average photosynthetically active radiation (PAR) of 88 ± 16 μmol m^{-2} s^{-1} during the illuminated period (Wilkie and Mulbry, 2002) using a bank of six fluorescent lamps (Philips, 40 W, Germany) located 15 cm over the phototrophic biofilm surface. The biofilm bioreactor B was partially covered to prevent light penetration and served as control in the evaluation of the influence of microalgae growth during biofilm-based wastewater treatment. The bioreactors were inoculated with 3 L of microalgal consortium (0.8 g TSS L^{-1}) and 0.5 L of activated sludge (9.1 g TSS L^{-1}).

The biofilm bioreactors were operated at hydraulic retention times (HRT) of 10.4, 5.2 and 3.1 d with a continuous internal effluent recycling (Watson Marlow M60617) of 4.2 L m^{-2} min^{-1} in the first five operational stages and 9.0 L m^{-2} min^{-1} in the last operational stage (Table 3) (Mulbry et al., 2008). The HRTs were chosen according to typical values reported for wastewater treatment in microalgae-bacteria systems such as HRAPs (Groeneweg et al., 1980; Wang et al., 1996; de Godos et al., 2009a). The wastewaters treated were maintained at 4 °C under magnetic agitation (200 rpm) during the entire experimentation. Six different operational conditions were tested along the 220 d of experimentation (Table 3). Ten times diluted centrate was initially treated in the biofilm bioreactors at 10.4 d of HRT in the two first stages (operated at an effluent recycling rate of 4.2 L m^{-2} d^{-1}), while domestic wastewater was subsequently treated at 10.4, 5.2 and 3.1 d of HRT in the third, fourth and fifth stage, respectively, also at an effluent recycling rate of 4.2 L m^{-2} d^{-1}. The carbon, nitrogen and phosphorus loads ranged from 0.6 to 4.9 g C m^{-2} d^{-1}, 0.4 to 1.7 g N m^{-2} d^{-1} and 0.1 to 0.34 g P m^{-2} d^{-1}, respectively. The influence of the effluent recycling rate on process performance was evaluated at 3.1 d of HRT in stage VI.

The temperature of the cultivation broth in the bioreactors, the dissolved O_2 concentration (DOC) and the influent and effluent flow rates were daily measured. The PAR was weekly monitored and the percentage of impinging light stored as chemical energy into biomass (photosynthetic efficiency, PE) was computed according to Eq. (1):

$$PE = \frac{W \cdot E}{G} \cdot 100 \quad (1)$$

Table 3
Operational conditions evaluated during the comparison of the bioremediation performance of the biofilm bioreactors.

Stage no.	Time (d)	Wastewater	Feed flow (L m^{-2} d^{-1})	Recirculation flow (L m^{-2} min^{-1})	HRT (d)	pH	C[a] (g m^{-2} d^{-1})	N[b] (g m^{-2} d^{-1})	P[c] (g m^{-2} d^{-1})
I	1–22	Diluted 1:10 centrate	6	4.2	10.4 ± 0.1	8.4 ± 0.1	0.6 ± 0.0	0.4 ± 0.0	0.05 ± 0.03
II	23–79	Diluted 1:10 centrate	6	4.2	10.4 ± 0.1	7.9 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.05 ± 0.04
III	80–134	Domestic wastewater	6	4.2	10.4 ± 0.1	7.4 ± 0.3	1.8 ± 0.0	0.6 ± 0.0	0.03 ± 0.02
IV	135–165	Domestic wastewater	12	4.2	5.2 ± 0.0	7.0 ± 0.0	3.7 ± 0.0	1.2 ± 0.0	0.08 ± 0.03
V	166–190	Domestic wastewater	20	4.2	3.1 ± 0.0	7.0 ± 0.0	4.9 ± 0.0	1.6 ± 0.1	0.12 ± 0.03
VI	191–220	Domestic wastewater	20	9.0	3.1 ± 0.0	7.0 ± 0.0	4.8 ± 0.0	1.7 ± 0.2	0.11 ± 0.01

[a] Total carbon (TC) loads were calculated considering the influent TOC and IC concentrations.
[b] Nitrogen loads were determined from the influent TN concentrations.
[c] Phosphorus loads corresponded to soluble phosphate concentrations.

where W represents the total harvested biomass in the cultivation surface (kg m^{-2} d^{-1}), E the specific heating value of the dry microalgal biomass (22.5 MJ kg^{-1} according to USDOE (1994) and Illman et al. (2000)) and G the impinging radiation (MJ m^{-2} d^{-1}). Liquid samples of 300 ml from the influent and effluent of both bioreactors were drawn twice a week to monitor the pH and the concentration of total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, total suspended solids (TSS) and volatile suspended solids (VSS). The carbon, nitrogen, phosphorous and suspended solid removal efficiency (RE) was calculated taking into account the corresponding empirical evaporation losses according to Eq. (2):

$$RE = \frac{(Q_{in} \cdot C_{in} - Q_{out} \cdot C_{out})}{Q_{in} \cdot C_{in}} \cdot 100 \quad (2)$$

where Q_{in} represents the influent flowrate (L d^{-1}); Q_{out} the effluent flowrate (L d^{-1}); and C_{in} and C_{out} are the influent and effluent concentrations of the target monitored parameters, respectively (mg L^{-1}).

The areal microalgal–bacterial biomass productivity was estimated by periodically harvesting the biomass from the cultivation surface of bioreactor A (Fig. 1) by mechanical scraping with a wood strip and from the settling chamber, while in bioreactor B biomass harvesting was carried out only twice over the 220 d of operation based on the negligible bacterial growth. The harvested biomass was dried for 24 h at 105 °C in a P-Selecta laboratory stove (SELECTA, Spain) prior to quantification.

2.4. Analytical procedure

TOC, IC and TN concentrations were determined using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1chemiluminescence module. N-NH_4^+ concentration was determined using an Orion Dual Star ammonia electrode (Thermo Scientific, The Netherlands). N-NO_3^-, N-NO_2^- and P-PO_4^{3-} concentrations were analyzed via HPLC–IC (Waters 432, conductivity detector, USA). All these analyses, including TSS and VSS determinations, were carried out according to Standard Methods (Eaton et al., 2005). A Crison microph 2002 (Crison instruments, Spain) was used for pH determination. DOC and water temperature were recorded using an OXI 330i oximeter (WTW, Germany). The PAR was measured with a Li-250A light meter (Li-COR Biosciences, Germany). The determination of the C and N content of the biomass was performed using a LECO CHNS-932 analyzer, while the analysis of its phosphorus content was carried out spectrophotometrically according to standard methods (Spectrophotometer U-2000, Hitachi, Japan).

3. Results and discussion

The low C/N/P ratio in both the centrate and domestic wastewater (Table 2), compared to the optimum reported C/N/P ratio for microalgae growth of 100/18/2, suggests a potential C limitation during wastewater treatment (Oswald, 1988). Despite different carbon, nitrogen and phosphorous REs were recorded depending on the type of wastewater and loading applied, the photobiofilm bioreactor always supported significantly higher nutrient removals (≈×2). The DOC was always higher than 3 mg O_2 L^{-1} regardless of the bioreactor and operational stage, which suggests that neither organic matter oxidation nor nitrification were limited by oxygen supply.

3.1. Centrate wastewater treatment

Stage I involved the start-up of the biofilm bioreactors and the tailoring of their design and operation. During this first period, the biofilm bioreactors treated 10 times diluted centrate at an HRT of 10.4 d. A thin and unstable algal–bacterial biofilm developed in bioreactor A due to its poor adherence and robustness, which resulted in a continuous biofilm detachment and subsequent biofilm floc disruption in the settling chamber. The low biodegradable TOC concentration available in the centrates, likely inducing a poor extracellular polymer formation, resulted in a poor microbial diversity and stability of the biofilm. A similar microalgae biofilm washout was reported by Boelee et al. (2011) during the tertiary treatment of a secondary effluent from an activated sludge process in an ATS. The illumination of the distribution and settling chambers also promoted the growth of suspended microalgae in bioreactor A during stage I, which resulted in negative TOC and TSS REs since a significant fraction of the inlet IC was photosynthetically transformed into particulate organic carbon in the form of suspended microalgae biomass (Figs. 2a and 3). Therefore, in order to prevent the growth of suspended microalgae, the distribution and settling chambers were covered with a black rubber from day 22 onwards. This modification constituted the main improvement carried out from the results obtained in the start-up period in both systems. Likewise, despite TOC and TSS removal efficiencies in bioreactor B were significantly higher than in bioreactor A, these REs were negligible or negative, concluding that both organic matter and suspended solid removals from centrate were not efficient during stage I regardless of the biofilm bioreactor tested (Figs. 2a and 3).

Stage II focused on the study of the different carbon and nutrient removal mechanisms from diluted centrate and on the comparison of the C, N and P removal efficiency in the algal–bacterial and bacterial biofilm bioreactors. The covering of the distribution and settling chambers initially resulted in enhanced TOC and TSS removal efficiencies in both bioreactors compared to those achieved at the end of stage I. However, a gradual decrease in the TOC and TSS removal capacity caused by the detachment and washout of the algal–bacterial biofilm in bioreactor A occurred approximately from day 56 onwards (Figs. 2a and 3). Likewise, a gradual deterioration in TOC and TSS removal was also recorded in bioreactor B. The average biomass harvested from bioreactor A surface during the first days of stage II was 0.5 g m^{-2} d^{-1}, which entailed a light utilization efficiency of 0.4%. This PE was lower than that reported by Ozkan et al. (2012) in a lab-scale algal biofilm photobioreactor (2.02 ± 0.17%) supplied with the autotrophic mineral salt medium BG-11. This low light utilization efficiency at the low light intensities here used suggests that another parameter such as carbon supply limited microalgae growth. At the end of stage II, the removal of TOC in the phototrophic bioreactor was negative and negligible in bioreactor B, while TSS removal in both bioreactors was approx. ≈0%.

The removal of inorganic carbon was almost complete at the end of stage II in both bioreactors, but while assimilation into biomass was likely the main IC removal mechanism in the algal–bacterial bioreactor, CO_2 stripping governed the fate of IC in the bacterial bioreactor since only 1.2 ± 0.5% of the initial IC was assimilated as nitrifying biomass. On the other hand, TN removal efficiencies decreased from 80% to 60% (corresponding to a decrease in the TN concentration removal from 62 to 41 mg N L^{-1}) in bioreactor A, with a complete NH_4^+ removal and an increase in the contribution of nitrification to NH_4^+ removal from 9% to 43%, which brought about a decrease in the pH from 7.5 to 6.5 during stage II. Likewise, TN removal decreased to 21% (14 mg N L^{-1}) in bioreactor B with a decrease in NH_4^+ RE to 86% and a contribution of nitrification to NH_4^+ removal of 63 ± 37%. Phosphorus removal decreased from 77% to 54% (9–8 mg P L^{-1}) in bioreactor A and remained below 21% (3 mg P L^{-1}) in bioreactor B, which confirmed once again the key role of biomass formation on C and nutrient removal in the algal–bacterial biofilm. The results here obtained

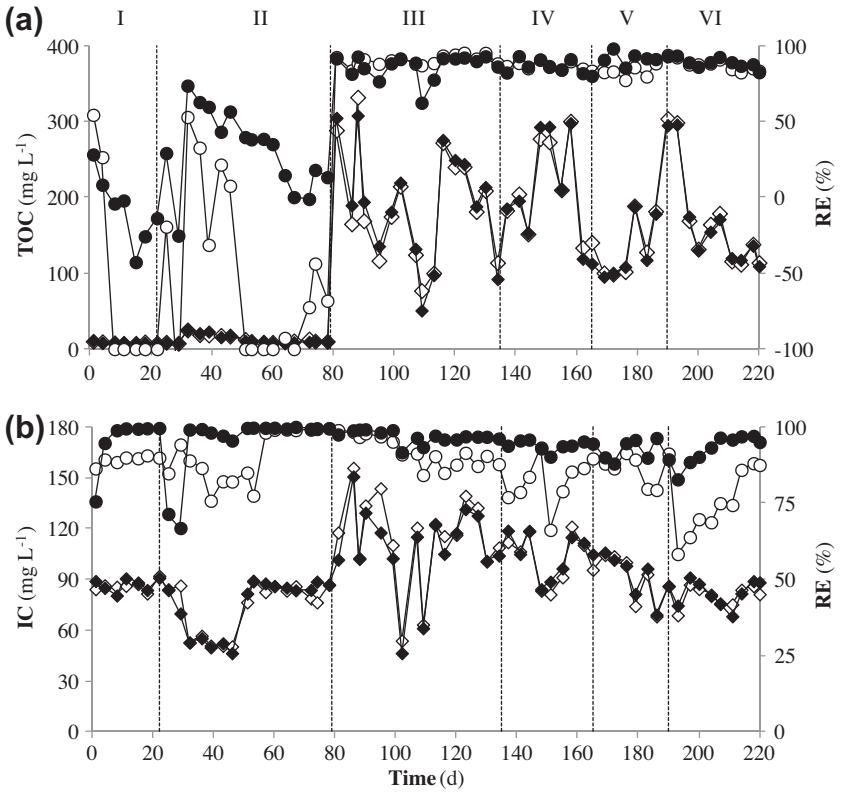

Fig. 2. Time course of the influent concentrations (diamonds) and removal efficiencies (circles) of TOC (a) and IC (b) during the treatment of diluted centrate and domestic wastewater in the biofilm bioreactor A (open symbols) and B (closed symbols).

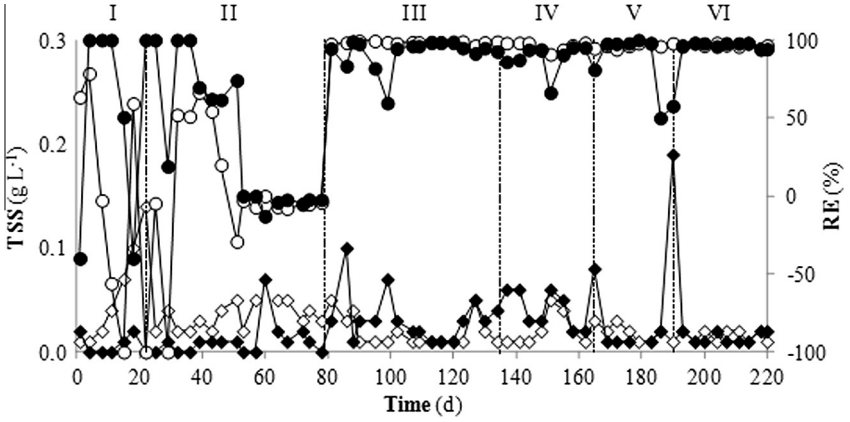

Fig. 3. Time course of the effluent concentrations (diamonds) and removal efficiencies (circles) of TSS during the treatment of diluted centrate (stages I and II) and domestic wastewater (stages III–VI) in the biofilm bioreactor A (open symbols) and B (closed symbols).

were in agreement with those reported in an ATS treating dairy manure at comparable N and P loads (≈0.64 g N m^{-2} d^{-1} and ≈0.08 g P m^{-2} d^{-1}), where lower TN–RE (40%) but higher TP–RE (69%) were recorded (Wilkie and Mulbry, 2002). Like in stage I, the poor adherence of the algal–bacterial biofilm together with the resuspension of the detached biomass in the settling chamber resulted in a negligible biomass harvested, whose composition was not possible to determine. Therefore, the contribution of stripping and assimilation to C, N and P removal was not quantified during stage II. The low biofilm adherence and the low growth rates of the algal–bacterial biofilm seem to confirm the hypothesis of a potential carbon limitation in both bioreactors, where besides the low carbon fraction originally present in the raw centrate (Table 2), part of it was removed by stripping in the bioreactors. Likewise, these results support the initial hypothesis of an efficient nutrient removal in the algal–bacterial biofilm during the treatment of diluted centrate.

The temperature of the cultivation broth is a critical parameter in biofilm activity and the thin water layer configuration in these bioreactors render them more sensitive to ambient temperature fluctuations than conventional suspended cultures (Murphy and Berberog, 2012). In addition, despite water temperatures in both bioreactors were similar and varied accordingly to the external temperature (data not shown), the evaporation losses in bioreactor A (2.5 ± 0.2 and 2.9 ± 0.4 L m^{-2} d^{-1} in stages I and II, respectively) were approximately two times higher than in bioreactor B (1.3 ± 0.3 and 1.2 ± 0.3 L m^{-2} d^{-1} in stages I and II, respectively), which can be explained by the partial covering of bioreactor B to avoid light penetration (Ozkan et al., 2012). The water evaporation losses here recorded during the 220 operational days were in

agreement with those reported by Murphy and Berberog (2012) (1–7.3 L m^{-2} d^{-1}).

3.2. Domestic wastewater treatment

During stage III the bioreactors were fed with primary settled domestic wastewater in order to elucidate whether wastewater treatment and the robustness of the biofilm were more effective for wastewaters with a higher carbon content in comparison with centrates. In addition, the different C, N and P removal mechanisms in period III were studied. The treatment of domestic wastewater at 10.4 d of HRT entailed higher carbon loads but similar nutrients loads (Table 3). TOC and IC removals in the algal–bacterial biofilm reactor accounted for 90 ± 3% and 91 ± 6% (corresponding to concentrations of 172 ± 27 and 105 ± 54 mg C L^{-1}, respectively), respectively, which corresponded to a removal of 1.7 ± 0.4 g C m^{-2} d^{-1}. However, only 50 ± 1% of the total C removed was recovered as algal–bacterial biomass. Similar removal efficiencies for TOC and IC were reached in the control bacterial biofilm (86 ± 8% and 97 ± 2%, respectively, corresponding to 171 ± 65 and 106 ± 26 mg C L^{-1}), where IC removal by stripping was the major C removal mechanism based on the absence of microalgae growth, the negligible contribution of IC assimilation into nitrifying biomass (1.8 ± 0.7% of the influent IC) and the low pH values. A robust algal–bacterial biofilm established in the cultivation surface of bioreactor A during stage III, concomitant with a complete TSS removal (Fig. 3). The average areal biomass harvested during this stage was 2 ± 1 g m^{-2} d^{-1}, which corresponded to a photosynthetic efficiency of 1.5%. These results confirmed the initial hypothesis that higher TOC biodegradable concentrations in the wastewater would support more robust biofilms and the fact that process performance was initially limited by carbon supply. Likewise, TSS removal in bioreactor B was almost complete despite the thin bacterial biofilm formed. Significant differences were recorded in the removal of nutrients in both bioreactors during stage III. Average TN removal efficiencies of 70 ± 8% and 36 ± 8% (corresponding to eliminations of 72 ± 13 and 37 ± 11 mg N L^{-1}) were recorded in the algal–bacterial and bacterial bioreactors, respectively. Despite the low share of NH_3 in the cultivation broth of bioreactor A (3.6% at pH ≈ 7.7), stripping was the main mechanism for TN removal since only 36 ± 15% of the TN removed was recovered as biomass. A similar nitrogen percentage (36 ± 11%) was recovered from diluted dairy manure as algal biomass in a laboratory ATS at N loading rates between 0.8 and 3.7 g m^{-2} d^{-1} (Kebede-Westhead et al., 2004). Nitrification accounted for 29 ± 7% and 62 ± 11% of the NH_4^+ removed in bioreactors A and B, respectively, with maximum NO_3^- concentrations in this experimental period of 58 and 83 mg N-NO_3^- L^{-1}, respectively. This high nitrification activity in the bacterial biofilm bioreactor decreased the pH to 5.2 by the end of stage III. In this context, while the low NH_4^+ concentrations in the algal–bacterial biofilm bioreactor likely limited the nitrification process, the presence of sufficiently high O_2 and NH_4^+ concentrations in bioreactor B suggest that either the low IC concentrations or the pH limited NH_4^+ oxidation in the bacterial biofilm (see Fig. 4).

The algal–bacterial biofilm exhibited P-PO_4^{3-} REs of 85 ± 9% (corresponding to a removal of 6 ± 3 mg P L^{-1}) in bioreactor A, while negative REs were recorded in the control bacterial biofilm reactor during stage III. Hence, while phosphorus removal in bioreactor A occurred via biomass assimilation (85 ± 13% of the P removed was recovered in the harvested biomass), the negative REs in bioreactor B were likely due to the hydrolysis of the biomass formed in the previous operational stages (hypothesis based on the absence of a significant biomass recovery in the control bioreactor). The phosphorus recovered as algal biomass in the current research was higher than the reported recoveries in a similar lab-scale ATS (34 ± 6%) treating dairy manure at phosphorus loading rates between 0.12 and 0.58 g P m^{-2} d^{-1} (Kebede-Westhead et al., 2004).

The results from stage III showed higher carbon and nutrient recoveries in the algal biomass harvested compared to those recorded during diluted centrate treatment at the same HRT. In this context, the higher carbon content of this wastewater likely mitigated the previous carbon limitation. Likewise, the satisfactory performance of the algal–bacterial biofilm for the recovery of nutrients from the domestic wastewater was confirmed by the higher nutrient removal recorded in the algal–bacterial biofilm compared to its bacterial counterpart. However, an HRT of 10 d is significantly higher than those typically used in conventional activated sludge processes. Therefore, the performance of the algal–bacterial biofilm bioreactor at lower HRTs (lower total bioreactor volumes for the same wastewater flowrate) must be evaluated.

The potential of both biofilm bioreactors was challenged by decreasing the HRT to 5.2 d (stage IV) and 3.1 d (stage V), respectively. The results obtained suggest that the same mechanisms discussed in stage III governed carbon and nutrient removal in stages IV and V. The algal–bacterial biofilm reactor supported TOC and IC removal efficiencies of 86 ± 3% and 81 ± 8% (corresponding to eliminations of 180 ± 29 and 84 ± 15 mg C L^{-1}), respectively, during stage IV, and 86 ± 6% and 85 ± 12% (corresponding to eliminations of 179 ± 15 and 78 ± 14 mg C L^{-1}), respectively, in stage V. The fact that only 48 ± 5% and 33 ± 5% of the total removed C was recovered as biomass in stages IV and V, respectively, suggest that stripping was the main C removal mechanism in the algal–bacterial biofilm. On the other hand, TOC and IC removal efficiencies of 87 ± 3% and 92 ± 4% (182 ± 20 and 87 ± 10 mg C L^{-1}), respectively, were recorded during stage IV in the bacterial biofilm, and 94 ± 2% and 91 ± 5% (183 ± 28 and 84 ± 10 mg C L^{-1}), respectively, during stage V. The high CO_2 gradients between the liquid and the gas phase (4.9 and 6.6 mg CO_2 L^{-1} in the liquid phase in stages IV and V *vs.* 0.31 mg CO_2 L^{-1} in equilibrium with the atmospheric CO_2 concentration) led to an intense IC removal by stripping. The carbon assimilated as nitrifying biomass was estimated to 0.8 ± 0.2% and 0.3 ± 0.2% of the inlet IC in stages IV and V, respectively, which confirmed the hypothesis of CO_2 stripping as the main C removal mechanism in bioreactor B. The algal–bacterial biomass harvested during both periods accounted for 3.1 and 2.6 g m^{-2} d^{-1} (corresponding to PEs of 2.6% and 2.2%, respectively), while a negligible biomass accumulation was observed in the bacterial biofilm. The high robustness of the biofilms formed in both bioreactors supported an efficient TSS removal (Fig. 3).

On the other hand, the TN removal in bioreactor A remained approximately constant in stages IV and V at 59 ± 11% and 54 ± 8% (corresponding to eliminations of 57 ± 12 and 42 ± 7 mg N L^{-1}), respectively. Surprisingly, NH_3 volatilization was likely the main removal mechanism in the algal–bacterial biofilm since only 37 ± 13% and 30 ± 15% of the TN removed was recovered as biomass. NH_4^+ was completely removed in the stages IV and V in the algal–bacterial biofilm, with nitrification accounting for 47 ± 11% and 78 ± 18% of the influent NH_4^+ in a process likely limited by the low NH_4^+ concentrations. The bacterial biofilm supported TN removals of 29 ± 6% and 34 ± 18% (corresponding to removals of 28 ± 7 and 29 ± 6 mg L^{-1}) in the periods IV and V, respectively, but an incomplete NH_4^+ removal in both stages. The decrease in the contribution of nitrification to the total NH_4^+ removal from stages IV to V (78 ± 18% and 53 ± 19%, respectively) could be attributed to the concomitant decrease in the influent NH_4^+ concentration. Likewise, phosphorus removal in stages IV and V during the operation of bioreactor A decreased to 57 ± 17% and 36 ± 22% (5 ± 1 and 2 ± 1 mg L^{-1}), respectively. Despite 90 ± 8% and 86 ± 10% of the total P removed was recovered in the harvested biomass, the lower phosphorus removals achieved com-

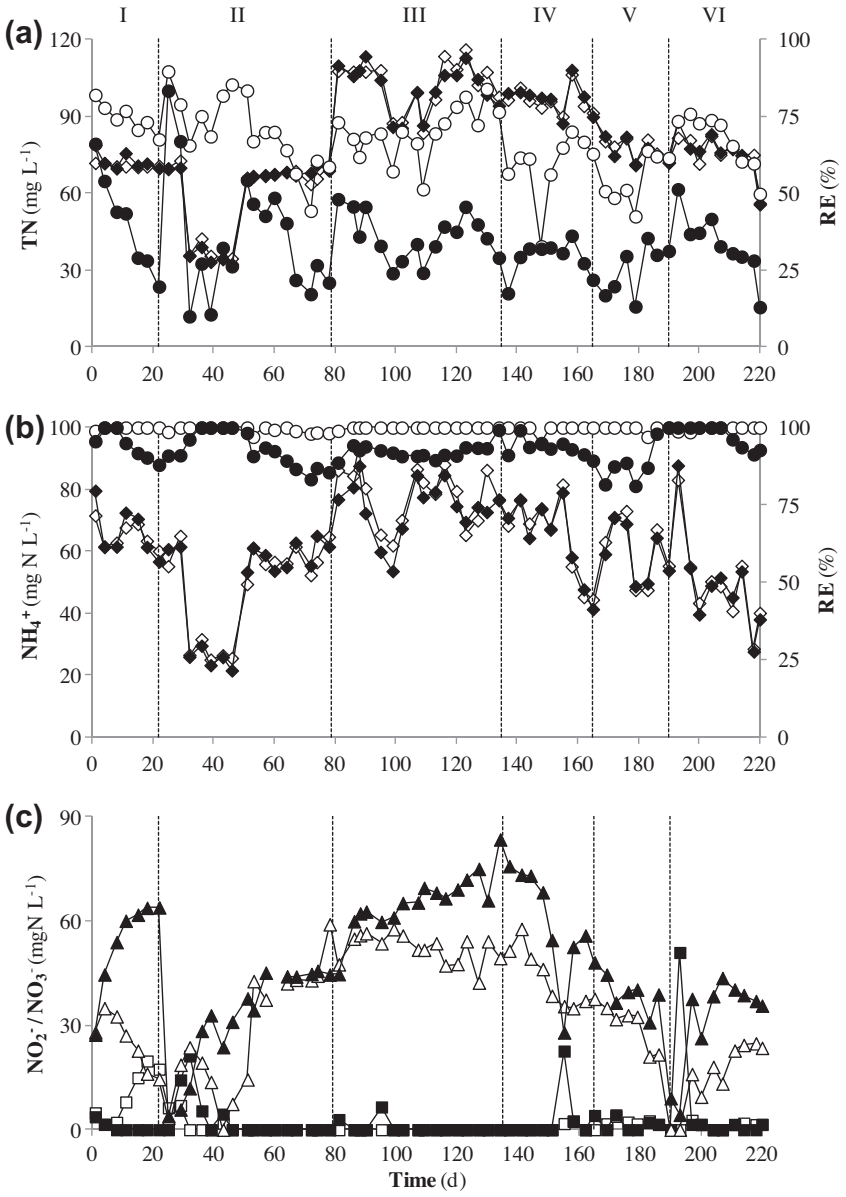

Fig. 4. Time course of the influent concentrations (diamonds) and removal efficiencies (circles) of TN (a), ammonium (b) and nitrite (squares)/nitrate (triangles) effluent concentrations during the treatment of diluted centrate and domestic wastewater in the biofilm bioreactor A (open) and B (closed).

pared to stage III were likely due to an overload of the biofilm purification capacity mediated by the high C, N and P loads, and the intensive C stripping from the system, which limited nutrient assimilation. In this context, a decrease in nutrient removal efficiencies at increasing loading rates was also reported in a benthic microalgae growth chamber (BAGC) treating dairy manure (Wilkie and Mulbry, 2002) (Table 1). Therefore, the lower HRTs tested did not result in an improvement in the carbon and nutrient removal efficiency in none of the bioreactor configuration tested. In this regard, the high HRTs in the algal–bacterial biofilm reactor for optimum biodegradation performance still involve the need for further developments targeting a significant decrease in the HRT to be able to compete with conventional wastewater treatment technologies such as activated sludge processes or UASB bioreactors.

In the final stage, the HRT was set at 3 d and the recirculation rate increased in order to assess any improvement in the biodegradation performance of the bioreactors as a result of a higher turbulence regime (see Fig. 5).

The increase in the effluent recycling rate up to 9 L m^{-2} min^{-1} in stage VI did not result in significant enhancements in process performance. Hence, the removal of TOC, TSS, IC, TN, NH_4^+ and PO_4^{3-} remained similar to those recorded in stage V in both biofilm reactors, and did not compensate the extra energy costs mediated by the increase of the effluent recycling rate. Similar results were recently reported by Higgins and Kendall (2012) in the evaluation of a life cycle energy of an ATS, who concluded that higher water recirculation rates result in a net increase in energy consumption without an enhancement in the ATS biomass productivity (g m^{-2} - d^{-1}). Despite a sudden decrease in NO_3^- concentrations at the end of stage V, the contribution of nitrification to NH_4^+ removal gradually recovered in stage VI in both bioreactors. The production of algal–bacterial biomass remained similar to that obtained in stage V (≈2.9 g m^{-2} d^{-1}), with an associated PE of 2.4%. C, N and P assimilation into the algal–bacterial biomass accounted for 30 ± 5%, 21 ± 13% and 102 ± 5% of their respective removals. The mechanical harvesting of the biomass in the algal–bacterial biofilm

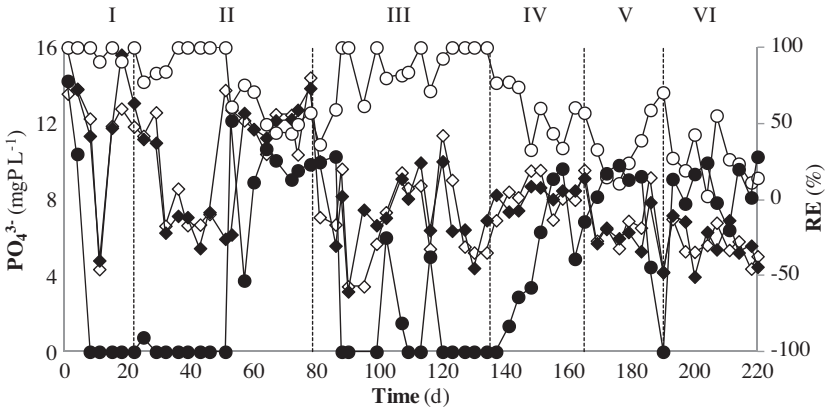

Fig. 5. Time course of the influent concentrations (diamonds) and removal efficiencies (circles) of phosphate during the treatment of diluted centrate and domestic wastewater in the biofilm bioreactor A (open symbols) and B (closed symbols).

Table 4
Dissolved oxygen concentration, evaporation losses, pH and temperature in the cultivation both of bioreactors A and B. N.M. = non monitoring.

Bioreactor	Stage no.	DOC (mg O_2 L^{-1})	Evaporation losses (L m^{-2} d^{-1})	pH	$Temperature_{WATER}$ (°C)
A	I	N.M.	2.5 ± 0.2	8.4 ± 0.3	20.0 ± 0.8
B			1.3 ± 0.3	6.7 ± 0.9	19.2 ± 1.2
A	II	7.4 ± 1.0	2.9 ± 0.4	7.5 ± 1.0	20.8 ± 1.9
B		6.9 ± 0.4	1.2 ± 0.3	6.3 ± 0.8	20.6 ± 1.7
A	III	5.5 ± 1.0	3.4 ± 0.5	7.7 ± 0.4	21.9 ± 2.2
B		4.7 ± 0.6	1.3 ± 0.7	5.8 ± 0.2	22.1 ± 2.1
A	IV	5.4 ± 0.8	4.3 ± 0.5	7.6 ± 0.2	24.1 ± 2.3
B		4.6 ± 0.6	1.9 ± 0.6	6.1 ± 0.4	24.4 ± 2.3
A	V	5.4 ± 0.7	4.2 ± 0.6	7.0 ± 0.2	25.3 ± 1.9
B		4.3 ± 0.7	2.5 ± 0.5	5.8 ± 0.6	25.6 ± 1.8
A	VI	5.8 ± 1.2	4.7 ± 0.8	7.2 ± 0.3	24.5 ± 1.7
B		4.5 ± 0.7	2.9 ± 0.3	6.4 ± 0.6	25.0 ± 2.0

bioreactor by scrapping the cultivation surface constitutes one of the main advantages of immobilized systems compared to suspended bioreactors due to its simplicity and low energy requirements. This harvesting method implies a significant decrease in the overall harvesting costs compared to conventional techniques such as centrifugation, filtration or coagulation/flocculation (de Godos et al., 2011), and even compared to innovative techniques based on the assistance of sludge in the cultivation broth in order to improve the biomass settleability in suspended systems (Su et al., 2012).

The results here obtained confirmed the initial hypothesis of a superior biodegradation performance of the algal–bacterial biofilms for the treatment of diluted centrates and primary domestic wastewaters, and highlighted the potential of this photobioreactor configuration as a core technology for domestic wastewater treatment in small communities. However, further developments focused on the optimization of the design and operation of this biofilm technology are necessary to outcompete conventional wastewater treatment technologies. Based on the results obtained, the evaluation of the performance of enclosed biofilm bioreactors (who avoid C and N removal by stripping and consequently would entail higher CO_2 and nutrient removals by assimilation into biomass) is mandatory.

The content of C, N and P in the algal bacterial biomass remained approximately constant during the 220 operational days, regardless of the type of wastewater treated and the loading rates at average values of 42 ± 2%, 7 ± 1% and 1.3 ± 0.3%, respectively. However, this empirical finding was not in agreement with those recorded by Boelee et al. (2011) and Mulbry et al. (2008), who observed an increase in the N and P content of the algal biomass harvested at increasing TN and TP loads.

The seasonal increase in ambient temperature promoted higher temperatures in the cultivation broth of the bioreactors and, consequently, the water evaporation losses in both bioreactors increased during stages IV and V compared to stage III (Tables 3 and 4). The increased turbulence in the cultivation broth as a result of the increased effluent recycling rate in stage VI caused also an increase in the water evaporation losses up to 4.7 ± 0.8 and 2.9 ± 0.3 L m^{-2} d^{-1}in the algal–bacterial and bacterial biofilm reactor, respectively. Despite water evaporation losses have been noticed in open systems, there is a lack of studies systematically evaluating the impact of water evaporation on the effluent carbon and nutrient concentrations (Mulbry et al., 2008; Murphy and Berberog, 2012). The high evaporation losses recorded in our experimental set-ups suggest that this high water footprint could eventually compromise the sustainability of open algal–bacterial biofilm bioreactors, and therefore, design and operational strategies must be developed in order to minimize this environmental impact.

4. Conclusions

This study confirmed the initial hypothesis of the superior performance in terms of C, N and P removal of algal–bacterial biofilm bioreactors compared to bacterial biofilm bioreactors, despite their high water footprint. Carbon removal in both bioreactors was similar and mainly due to stripping, regardless of the target wastewater and operational conditions. However, the algal–bacterial biofilm exhibited twice higher nutrient removal rates compared to the bacterial biofilm, where no significant phosphorous removal was recorded and stripping was the main nitrogen removal mechanism. The robustness of the treatment in terms of biofilm structural stability was highly influenced by the wastewater nature.

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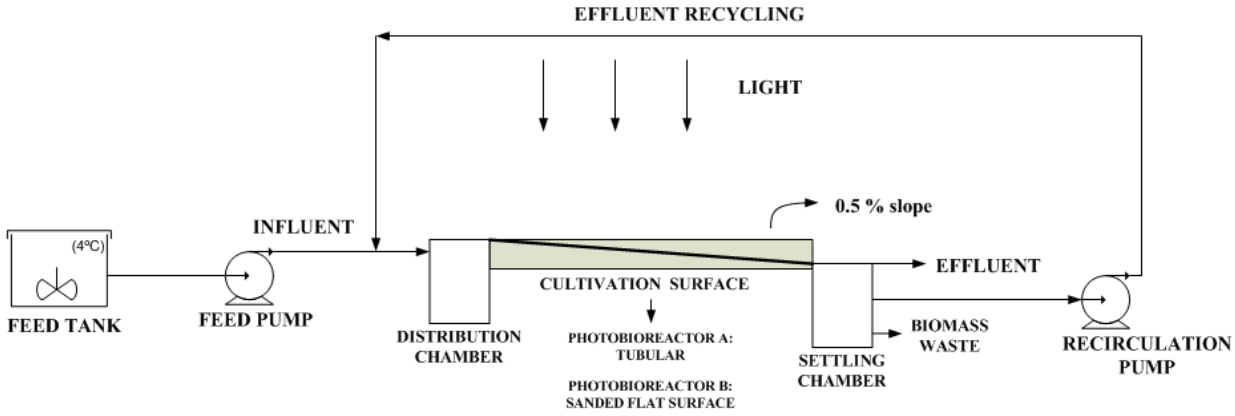
Chapter 5



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Enclosed tubular and open algal–bacterial biofilm photobioreactors for carbon and nutrient removal from domestic wastewater

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ABSTRACT

The mechanisms underlying carbon and nutrient removal from domestic wastewater in an enclosed tubular and an open biofilm photobioreactors were comparatively evaluated at hydraulic retention times (HRTs) of 10, 7 and 5 d, and internal recirculation rates of 4.2 and 9 L m^{-2} min^{-1}. Similar organic carbon removal efficiencies were recorded in both photobioreactors (63–97%) regardless of the operational conditions, while a superior inorganic carbon removal was always achieved in the open biofilm photobioreactor (≈100%). Nitrogen and phosphorous removal decreased in both photobioreactors when decreasing the HRT to 7 and 5 d, phosphorus being only efficiently removed in the open photobioreactor. Maximum organic carbon, nitrogen and phosphorus removals of 89 ± 2%, 92 ± 5% and 96 ± 2%, respectively, were achieved in the open biofilm photobioreactor at a HRT of 10 d. Assimilation into algal–bacterial biomass accounted for most nitrogen and phosphorous removal in both photobioreactors and for carbon removal in the tubular photobioreactor, while stripping (as a result of the low pHs mediated by an intense NH_4^+ nitrification) was responsible for most inorganic carbon removal in the open system. No significant differences in the carbon, nitrogen and phosphorus content of the harvested biomass were recorded regardless of the photobioreactor configuration and nutrient loading rates. Finally, the monitoring of the dynamics of microalgae population revealed that open biofilm photobioreactors can support a higher microalgae diversity than their enclosed counterparts.

1. Introduction

The ever-increasing generation of domestic wastewaters worldwide, together with the high aeration costs and poor nutrient removals of conventional wastewater treatment technologies such as activated sludge and anaerobic digestion, respectively, have promoted the development of alternative cost-effective wastewater treatment methods (Bhatnagar and Sillanpää, 2010; Mata et al., 2012). In this regard, algal–bacterial processes constitute a low-cost and environmentally friendly platform technology able to overcome the above mentioned limitations (Muñoz and Guieysse, 2006). The symbiosis between microalgae and bacteria is supported by the production of O_2 by microalgae in the presence of light and CO_2. This photosynthetic oxygen is in situ used by heterotrophic bacteria to oxidize the organic pollutants present in the wastewater, producing in turn the CO_2 required for microalgal photosynthesis (Muñoz and Guieysse, 2006). This free oxygenation of the process, together with the low capital investment for photobioreactors' installation and the simplicity of their operation and maintenance, make microalgae-bacteria systems cheaper than activated sludge processes (Craggs et al., 2011). The higher biomass productivity potential of algal–bacterial systems compared to activated sludge processes often entails an enhanced nutrient recovery. Thus, the simultaneous assimilation into biomass of both the CO_2 released by the oxidation of the total organic carbon (TOC) and the inorganic carbon (IC) present in the influent wastewater often

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results in higher biomass productivities (Posadas et al., 2013). Therefore, the production of a valuable biomass could eventually offset part of the operating costs of the treatment process (Tredici, 2004).

The first applications of this green technology were carried out in the mid 1950s in California for the treatment of domestic wastewaters in high rate algal ponds (HRAPs) (Oswald, 1988). However, despite a successful removal of both organic matter and nutrient has been consistently achieved during the treatment of domestic, industrial and livestock wastewaters in HRAPs over the past 50 years (De Godos et al., 2009a; García et al., 2000; Sevrin-Reyssac, 1998), microalgae harvesting still constitutes one of the main techno-economic limitations of this technology (Park et al., 2011). A large number of microalgae harvesting techniques based on chemical (De Godos et al., 2011), physical (Uduman et al., 2010) or biological (Lee et al., 2008) processes have been developed to date, but the contamination of the treated effluent with chemicals or their high energy requirements have hindered the development of a universal single method for microalgae harvesting (Christenson and Sims, 2011).

In this regard, microalgae immobilization has the potential to enhance microalgae biomass recovery from the treated wastewaters (Ruiz-Marín et al., 2010). However, the high costs and lack of long-term structural stability of conventional microalgae immobilization matrices (e.g. carrageenan, chitosan, or alginate) (Muñoz et al., 2009) often compromise the economic viability of conventional microalgae-immobilization techniques (Christenson and Sims, 2011). Biofilm photobioreactor configurations based on the natural growth of microalgae attached onto the wall or surface of the photobioreactors could eventually overcome the above limitations, while supporting a cost-effective separation of the microalgal detached flocs by sedimentation (Zamalloa et al., 2013). Preliminary studies assessing the potential of this innovative immobilization approach showed promising carbon and nutrient removals during the treatment of domestic wastewaters (Boelee et al., 2011; Posadas et al., 2013; Zamalloa et al., 2013) and livestock effluents (Craggs et al., 1996; De Godos et al., 2009a; Kebede-Westhead et al., 2004; Wilkie and Mulbry, 2002). For instance, Posadas et al. (2013) demonstrated the superior nutrient removal capacity of algal–bacterial biofilms (≈75% for total nitrogen and ≈80% for total phosphorous at 10 days of hydraulic residence time (HRT)) compared to bacterial biofilms during the secondary treatment of domestic wastewaters. However, approximately 50 and 60% of the total carbon and nitrogen removed in open algal–bacterial photobioreactors, respectively, occurred by stripping. The occurrence of carbon limitations during wastewater treatment in algal–bacterial photobioreactors is well documented in literature (Craggs et al., 2011), and significantly reduces both biomass productivity and nutrient removal capacity. In addition, the high water footprint (0.5–7 L m^{-2} d^{-1}) of open algal–bacterial biofilm photobioreactors might also compromise the environmental sustainability of this technology (Murphy and Berberog, 2012; Posadas et al., 2013). Therefore, the development of enclosed algal–bacterial biofilm photobioreactors is mandatory in order to mitigate both carbon and nitrogen losses by stripping, and to reduce the water footprint of the process.

In this work, the performance of an open and an enclosed tubular algal–bacterial biofilm photobioreactors was comparatively evaluated in terms of carbon and nutrient removal from domestic wastewater at different hydraulic loading rates, internal recirculation rates and pHs. The mechanisms of C, N and P removal in each photobioreactor configuration, along with the carbon, nitrogen and phosphorus content of the biomass generated, were quantified at each operational stage. Finally, the influence of the biofilm configuration on the dynamics of microalgae population was investigated.

2. Materials and methods

2.1. Microorganisms and culture conditions

The enclosed tubular and the open biofilm photobioreactors were inoculated with a mixed microalgal–bacterial consortium obtained from an open algal–bacterial biofilm bioreactor treating domestic wastewater and from the paddlewheel of a pilot HRAP treating diluted centrates, both located at the Department of Chemical Engineering and Environmental Technology (University of Valladolid, Spain).

2.2. Domestic wastewater

Fresh raw domestic wastewater was daily collected from a public sewer nearby the Department of Chemical Engineering and Environmental Technology of Valladolid University. The domestic wastewater was pre-treated by screening and primary sedimentation (3 h of retention time) and its composition corresponded to a medium-strength wastewater (Table 1).

2.3. Experimental set-up

The experimental set-up consisted of an enclosed tubular and an open biofilm photobioreactors composed of 2 chambers as distribution and settling units (Fig. 1). The total volume of the systems was 31 L distributed in the above mentioned chambers of 15.5 L of individual volume and a connecting cultivation surface of 0.5 m^2 with a slope of 0.5% (Craggs et al., 1996). The cultivation surface in the enclosed tubular algal–bacterial photobioreactor consisted of 35 methacrylate tubes (10 mm internal diameter × 1 mm thickness × 1 m long; Posten, 2009) in order to allow for an optimal light distribution. The cultivation surface in the open algal–bacterial photobioreactor consisted of a foam PVC flat plate (1 m long × 0.5 m wide) sanded in order to promote microbial attachment. The maximum water layer in the cultivation surface of the open photobioreactor was 0.5 cm (Posadas et al., 2013). Both the pre-treated domestic wastewater and effluent recirculation entrances were located in the distribution chamber. The mixture of wastewater and recycled effluent was distributed homogeneously from the distribution chamber along the cultivation surface and collected in the settling chamber, where both the effluent and recirculation intake were located (Fig. 1).

Both photobioreactors were exposed to a light:dark regime of 16:8 h:h at an average photosynthetically active radiation (PAR) of 74 ± 3 μmol m^{-2} s^{-1} during the illuminated period using a bank of six fluorescent lamps (Philips, 40 W, Germany) located 15 cm over the cultivation surfaces. Each photobioreactor was inoculated with 2 L of a mixed microalgae–bacterial consortium (5.7 g TSS L^{-1}) and operated at HRTs of 10, 7 and 5 d using a continuous internal effluent recycling (Watson Marlow M60617) of 4.2 L m^{-2} min^{-1} in the first 3 operational stages and 9.0 L m^{-2} min^{-1} in the last two periods (Table 2). The HRTs corresponded to typical values reported for domestic wastewater treatment in microalgal–bacterial systems such as HRAPs (Olguín, 2003) or open biofilm photobioreactors (Posadas et al., 2013). The treated wastewaters were maintained at 4 °C under magnetic agitation (200 r.p.m.) during the entire experimentation.

The carbon, nitrogen and phosphorus loading rates in the three first operational stages ranged from 1.7 to 3.5 g C m^{-2} d^{-1}, 0.7 to 1.2 g N m^{-2} d^{-1} and 0.08 to 0.13 g P m^{-2} d^{-1}, respectively. The influence of an increase in the effluent recycling rate up to 9.0 L m^{-2} d^{-1} on process performance and biomass harvesting efficiency was evaluated in stage IV, while in stage V the pH of the cultivation broth of the photobioreactors was increased and maintained at 9.2 ± 0.2

Table 1
Composition of the pre-treated domestic wastewater.

TOC ($mg\,L^{-1}$)	IC ($mg\,L^{-1}$)	TN ($mg\,L^{-1}$)	NH_4^+ ($mg\,N\,L^{-1}$)	PO_4^{3-} ($mg\,P\,L^{-1}$)	C:N:P ratio	TSS ($g\,L^{-1}$)	pH
167 ± 64	122 ± 15	106 ± 9	86 ± 15	12 ± 3	100:37:5	0.19 ± 0.08	7.5 ± 0.2

Neither NO_2^- nor NO_3^- were detected in the domestic wastewater.

Table 2
Operational conditions evaluated during the comparison of the bioremediation performance of the enclosed tubular and the open biofilm photobioreactors.

Stage	Time (d)	HRT (d)	Recirculation flow rate ($L\,m^{-2}\,min^{-1}$)	pH	C[a] ($g\,m^{-2}\,d^{-1}$)	N[b] ($g\,m^{-2}\,d^{-1}$)	P[c] ($g\,m^{-2}\,d^{-1}$)
I	1–40	10	4.2	7.6 ± 0.2	1.7 ± 0.3	0.65 ± 0.04	0.08 ± 0.03
II	41–89	7	4.2	7.6 ± 0.3	2.5 ± 0.7	0.90 ± 0.06	0.11 ± 0.03
III	90–120	5	4.2	7.3 ± 0.1	3.5 ± 0.5	1.23 ± 0.13	0.13 ± 0.01
IV	121–127	5	9.0	7.6 ± 0.3	2.7 ± 0.1	1.12 ± 0.11	0.10 ± 0.00
V	128–143	5	9.0	7.6 ± 0.2	1.8 ± 0.6	0.84 ± 0.26	0.08 ± 0.05

[a] Total carbon (TC) loading rates were calculated considering the influent TOC and IC concentrations.
[b] Nitrogen loading rates were determined from the influent TN concentrations.
[c] Phosphorus loading rates corresponded to soluble phosphate concentrations.

in order to prevent carbon removal by stripping (Posadas et al., 2013). Despite process operation in stages IV and V occurred at 5.0 d of HRT, the carbon, nitrogen and phosphorus loading rates were lower than in stage III (Table 2) due to the high seasonal variability of the domestic wastewater characteristics.

Temperature and dissolved O_2 concentration (DO) in the cultivation broth of the photobioreactors, and the influent and effluent flow rates were daily measured. Liquid samples of 300 mL from the influent and effluent of both photobioreactors were drawn twice a week to monitor the pH and the concentration of TOC, IC, total nitrogen (TN), NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, total suspended solids (TSS) and volatile suspended solids (VSS). The carbon, nitrogen, phosphorous and suspended solid removal efficiency (RE) was calculated considering the corresponding empirical evaporation losses according to equation (1):

$$RE = \frac{(Q_{in} \cdot C_{in} - Q_{out} \cdot C_{out})}{Q_{in} \cdot C_{in}} \times 100 \quad (1)$$

where Q_{in} represents the influent flowrate ($L\,d^{-1}$); Q_{out} the effluent flow rate ($L\,d^{-1}$); and C_{in} and C_{out} correspond to the influent and effluent concentrations of the target monitored parameters, respectively ($mg\,L^{-1}$).

The PAR was weekly monitored and the percentage of impinging light stored as chemical energy into biomass (photosynthetic efficiency, PE) was computed according to equation (2):

$$PE = \frac{W \cdot E}{G} \cdot 100 \quad (2)$$

where W represents the areal microalgal–bacterial biomass productivity ($kg\,m^{-2}\,d^{-1}$), E is the specific heating value of the dry microalgal–bacterial biomass (22.5 $MJ\,kg^{-1}$ according to Illman et al., 2000) and G is the impinging irradiation ($MJ\,m^{-2}\,d^{-1}$).

The areal biomass productivity was estimated by periodically harvesting the biomass from the settler in photobioreactor A and by both mechanical scraping and harvesting from the settling chamber in photobioreactor B. The harvested biomass was dried for 24 h at 105 °C in a P-Selecta laboratory stove (SELECTA, Spain) prior to quantification. Microalgal biofilm samples were also harvested at the steady state of each operational stage to monitor the dynamics of microalgae population.

2.4. Analytical procedures

TOC, IC and TN concentrations were determined using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. N-NH_4^+ concentration was determined using an Orion Dual Star ammonia electrode (Thermo Scientific, The Netherlands), while N-NO_3^-, N-NO_2^- and P-PO_4^{3-} concentrations were analyzed via HPLC-IC (Waters 432, conductivity detector, USA). All these analyses, including TSS and VSS determinations, were carried out according to Standard Methods (Eaton et al., 2005). An Eutech Cyberscan pH510 (Eutech instruments, The Netherlands) was used for pH determination. DO and temperature were recorded using an OXI 330i oximeter (WTW, Germany). The PAR was measured with a LI-250A light meter (LI-COR Biosciences, Germany). The determination of the C and N content of the algal–bacterial biomass was performed using a LECO CHNS-932 analyzer, while the phosphorus content analysis was carried out spectrophotometrically according to Standard Methods (Spectrophotometer U-2000, Hitachi, Japan). The identification,

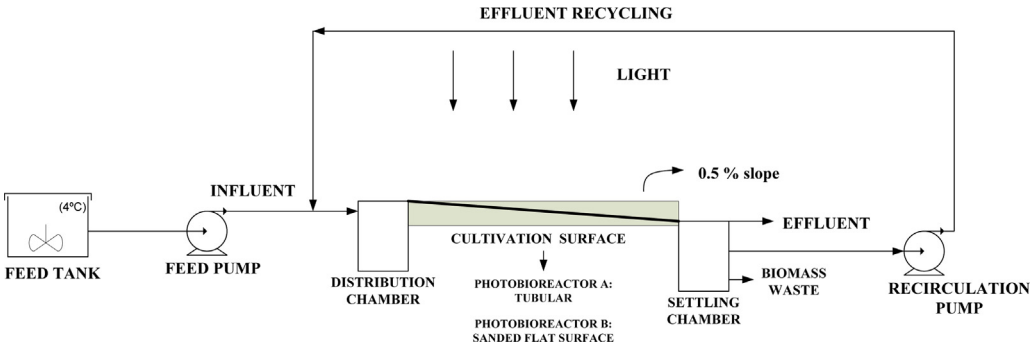

Fig. 1. Schematic diagram of the laboratory-scale tubular and open biofilm photobioreactors.

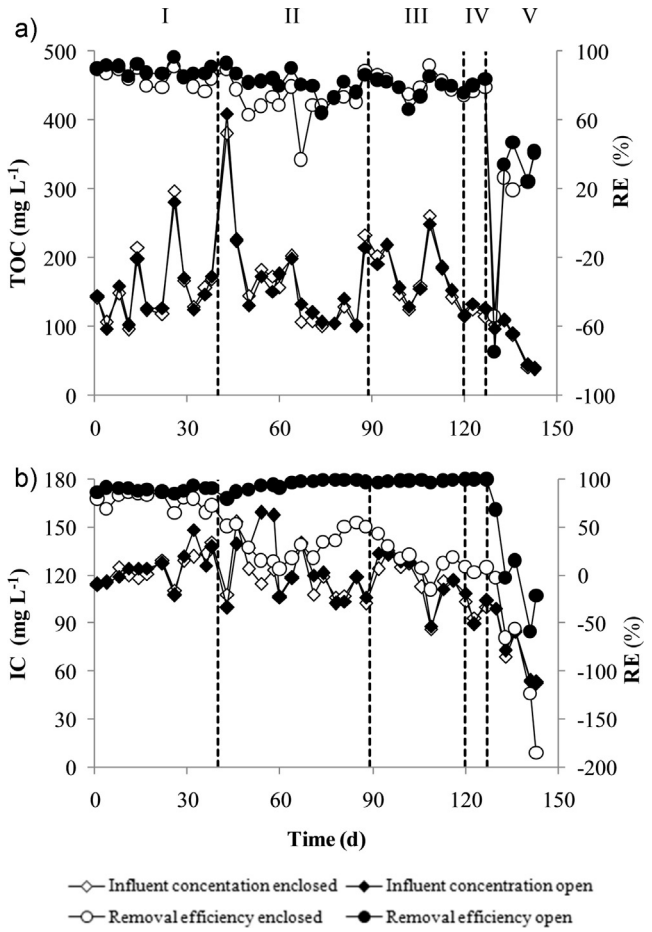

Fig. 2. Time course of the influent concentrations and removal efficiencies of TOC (a) and IC (b) during the treatment of domestic wastewater in the enclosed tubular photobioreactor and in the open biofilm photobioreactor.

quantification and biometry measurements of microalgae and cyanobacteria (from now on referred as microalgae) were carried out by microscopical examination (OLYMPUS IX70, USA) of microalgal samples (fixed with lugol acid at 5% and stored at 4 °C prior to analysis) according to Sournia (1978).

3. Results and discussion

The low C/N/P ratio of the domestic wastewater after primary treatment (100/37/5) suggested a potential occurrence of C limitation during wastewater treatment in both photobioreactors based on the optimum (100/18/2) C/N/P ratio reported for microalgae growth (Oswald, 1988) and previous experimental findings in our lab (Posadas et al., 2013). Despite the high variability in the composition of the domestic wastewater (Table 1), the tubular and the open algal–bacterial biofilm photobioreactors tested exhibited a high robustness, likely due to the high operational resilience associated to the high HRTs (10, 7 and 5 d).

Overall, the open algal–bacterial photobioreactor exhibited higher C, N and P removals than its enclosed counterpart at the HRTs tested (Figs. 2–4). The low DOs recorded in photobioreactor A ($\leq$1 mg O_2 L^{-1} from stage II onward) (Table 3) likely limited both organic matter oxidation (which requires DO > 0.5 mg O_2 L^{-1}) and NH_4^+ nitrification (which requires DO > 2 mg O_2 L^{-1}) (Table 3) (Metcalf and Eddy, 2003), situation never encountered in the open photobioreactor due to the active O_2 exchange between the recirculation liquid and the atmosphere. The recorded temperatures

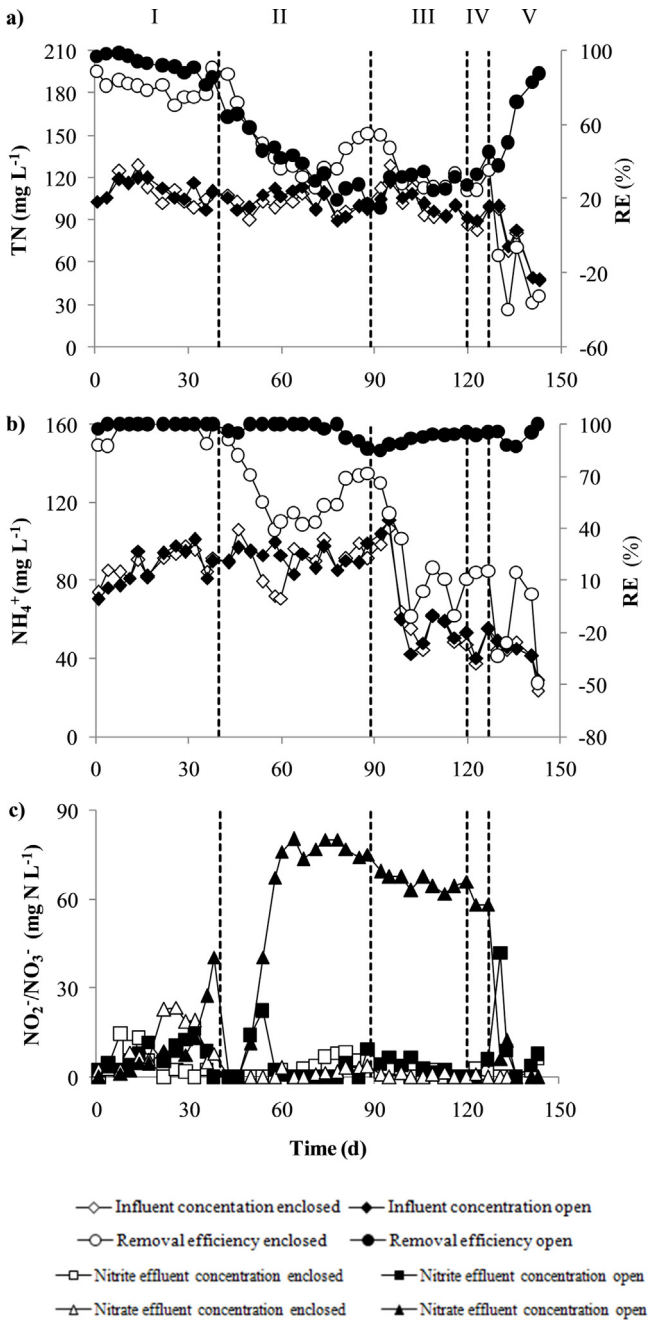

Fig. 3. Time course of the influent concentrations and removal efficiencies of TN (a) and ammonium (b), and nitrite/nitrate effluent concentrations (c) during the treatment of domestic wastewater in the enclosed tubular photobioreactor and in the open biofilm photobioreactor.

(20–25 °C) supported an adequate biological activity in both photobioreactors (Table 3) (Mata et al., 2012).

3.1. Influence of the HRT on wastewater treatment

A robust and stable algal–bacterial biofilm established during stage I in both photobioreactors after 8 days of operation. The high adherence of the biomass to the tubes resulted in a negligible biomass detachment and, subsequently, in no biomass recovery in the settler. The average algal–bacterial biomass harvested from photobioreactor B surface during stage I accounted for 3.8 ± 0.4 g m^{-2} d^{-1}, which corresponded to a PE of 3.2 ± 0.3%. These

Table 3
Dissolved oxygen concentration, evaporation losses, pH and temperature in the cultivation broth of photobioreactors A and B.

Photobioreactor	Stage	DO (mg O_2 L^{-1})	Evaporation losses (L m^{-2} d^{-1})	pH	Temperature (°C)
A	I	3.7 ± 2.2	[a]	7.8 ± 0.3	24.0 ± 2.0
B		7.3 ± 0.8	3.5 ± 0.4	8.3 ± 0.2	21.7 ± 1.9
A	II	0.5 ± 0.2	[a]	7.8 ± 0.2	22.2 ± 2.0
B		5.5 ± 0.9	3.3 ± 0.3	7.3 ± 0.6	20.1 ± 1.8
A	III	0.3 ± 0.2	[a]	7.7 ± 0.1	23.0 ± 1.9
B		4.0 ± 0.4	3.7 ± 0.3	6.7 ± 0.2	20.7 ± 1.9
A	IV	0.3 ± 0.1	[a]	7.7 ± 0.1	24.5 ± 1.0
B		4.0 ± 0.0	3.9 ± 0.5	6.3 ± 0.3	22.1 ± 1.3
A	V	0.2 ± 0.1	[a]	9.2 ± 0.1	24.6 ± 1.4
B		4.2 ± 0.6	4.0 ± 0.4	9.2 ± 0.1	22.6 ± 1.6

[a] Enclosed system.

PEs and biomass productivities were comparable to those reported by Mulbry et al. (2008) in a laboratory algal turf scrubber (ATS) treating dairy and swine manure effluent. In this context, the low biomass productivities here obtained at this high PE, together with the almost complete organic carbon and inorganic depletion, suggested that carbon rather than light supply limited the performance of the open photobioreactor in stage I (Posadas et al., 2013).

The DO in photobioreactors A and B decreased during stage I from 9.03 and 8.11 mg O_2 L^{-1} to 1.85 and 6.90 mg O_2 L^{-1}, respectively. This severe DO decrease in the enclosed tubular photobioreactor was likely mediated by the active microbial O_2 consumption as a result of the oxidation of organic matter and NH_4^+, and the absence of O_2 exchange with the atmosphere (Singh and Sharma, 2012). The TOC-RE in photobioreactor A accounted for 85 ± 5% and was comparable to the TOC-RE in photobioreactor B (89 ± 3%) (Fig. 2a). On the other hand, the IC removal in photobioreactor A reached 78 ± 8% (Fig. 2c) and was mainly caused by IC assimilation by microalgae since a low nitrification activity (which would represent a consumption of ≈0.8 ± 0.6% of the total influent IC) was recorded in this system during stage I as a result of the low DOs. CO_2 concentrations in the bulk liquid phase in the open photobioreactor were estimated considering the dissociation of the different carbon species in aqueous solution according to the different equilibria ($CO_{2(l)} + H_2O_{(l)} \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$; pKa_1 ($H_2CO_3 \leftrightarrow HCO_3^-$) = 6.35; pKa_2 ($HCO_3^- \leftrightarrow CO_3^{2-}$) = 10.33) (Metcalf and Eddy, 2003) and the corresponding pH and IC concentrations in the photobioreactor. The estimated CO_2 concentrations in the bulk liquid phase (0.5 ± 0.1 mg L^{-1}) were similar to the aqueous CO_2 concentration (0.3 mg L^{-1}) in equilibrium with atmospheric concentration, which suggest that most IC was removed by assimilation into biomass during stage I despite IC stripping occurred.

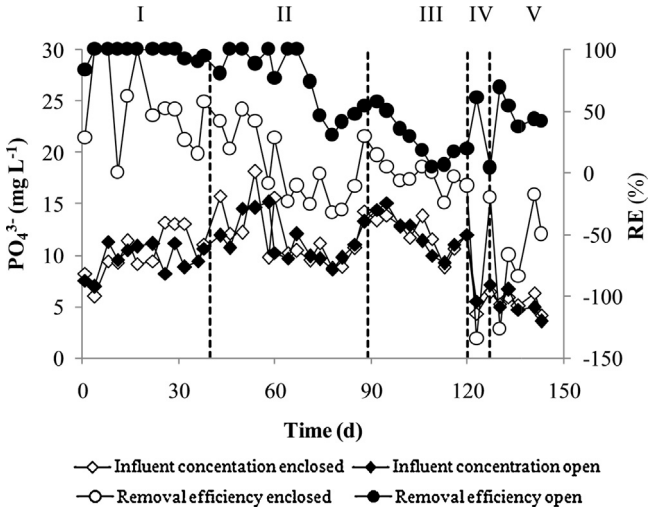

Fig. 4. Time course of the influent concentrations and removal efficiencies of phosphate during the treatment of domestic wastewater in the enclosed tubular photobioreactor and in the open biofilm photobioreactor.

Photosynthesis maintained the pH at ≈7.8 ± 0.3 (Table 3). On the other hand, IC-RE in photobioreactor B represented 89 ± 2% (Fig. 2b), with assimilation into biomass being the main carbon removal mechanism (at average pHs of 8.3 ± 0.2) since approximately 100% of the total carbon removed was recovered in the harvested biomass in stage I. Likewise, the IC consumed in the nitrification process (corresponding to a nitrification share of 14.9 ± 10.9%) accounted for 0.8 ± 0.6% of the inlet IC.

Nitrogen removal in photobioreactor A occurred mainly by assimilation into biomass in stage I and accounted for TN-REs of 80 ± 6% (Fig. 3a), which confirmed the potential of enclosed biofilm photobioreactors for nitrogen recovery based on the absence of NH_4^+ volatilization (De Godos et al., 2009b). NH_4^+ was completely consumed with a maximum contribution of nitrification to NH_4^+ removal of 28%, which corresponded to the highest effluent NO_3^- concentrations (23 mg N-NO_3^- L^{-1}) (Fig. 3c) in photobioreactor A over the entire operational period. The removal of TN in the open algal–bacterial biofilm bioreactor was slightly higher than in the enclosed tubular reactor (92 ± 5%), likely due to the contribution of NH_3 stripping since only 56.3 ± 4.7% of the TN removed from the wastewater was recovered in the harvested biomass. These results were in agreement with previous studies reporting the significant contribution of N-NH_4^+ stripping to nitrogen removal in open algal systems. For instance, García et al. (2000) recorded N-NH_4^+ removals by volatilization of up to 60% in a HRAP treating urban wastewater at 10 d of HRT.

The tubular and the open algal–bacterial biofilm photobioreactors exhibited P-PO_4^{3-} REs of 68 ± 18% and 96 ± 2%, respectively (Fig. 4). Phosphorus removal occurred via assimilation into biomass since the recorded pHs in both systems were lower than 8.6 and P precipitation was not likely to occur at such low pHs (Cai et al., 2013). In this context, 87 ± 12% of the total phosphorus removed was recovered in the harvested biomass in the open biofilm bioreactor.

Total solid REs remained approximately at 100% due to the high adherence of the algal–bacterial biomass onto both photobioreactor's surfaces, with average effluent TSS concentrations of 0.01 g TSS L^{-1} (Fig. 5). These TSS concentrations were lower than the maximum admissible TSS concentration for discharge of treated domestic wastewaters (0.035 g TSS L^{-1}) according to the 2000/60/CE Directive.

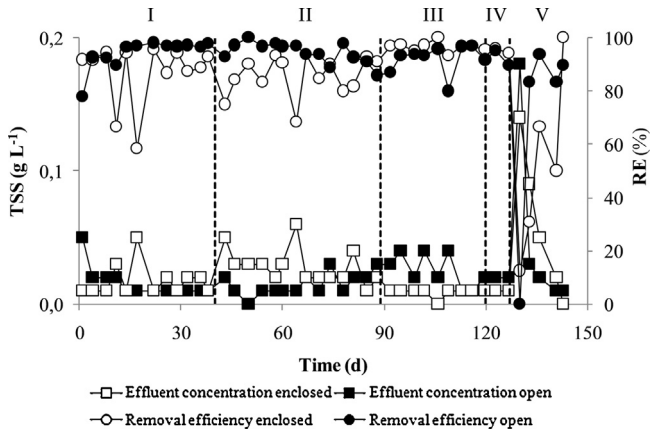

Fig. 5. Time course of the effluent concentrations and removal efficiencies of TSS during the treatment of domestic wastewater in the enclosed tubular photobioreactor and in the open biofilm photobioreactor.

The mechanisms governing TOC and NH_4^+ oxidation in the enclosed and the open biofilm photobioreactors here hypothesized, and which likely determined photobioreactor performance, were influenced by the location of the active microalgae population in the photobioreactor (Fig. 6a and b). Thus, while in the enclosed system the O_2 flux originated at the inner part of the biofilm and diffused counter-currently with TOC and NH_4^+ fluxes, in the open biofilm the photosynthetic O_2 flux diffused co-currently with TOC, NH_4^+ and atmospheric O_2 fluxes (De Godos et al., 2009b).

Despite the satisfactory carbon and nutrient removal performance of the algal–bacterial biofilm in both photobioreactor configurations, the HRT was decreased to 7 and 5 d in stages II and III, respectively, in order to compare their maximum bioremediation potential under laboratory conditions and identify process limitations at higher loading rates. Hence, the increase in C, N and P loading rates in stages II and III resulted in negligible DOs in the enclosed photobioreactor regardless of the operational stage and a decrease in the DO in the open biofilm to 5.5 ± 0.9 and 4.0 ± 0.4 mg O_2 L^{-1}, respectively (Table 3). Photobioreactor A supported TOC and IC removal efficiencies during stage II of $71 \pm 12\%$ and $33 \pm 17\%$, respectively, and $81 \pm 6\%$ and $20 \pm 12\%$, respectively, during stage III. The low IC removal performance, together with the low DOs recorded, indicated that wastewater treatment in photobioreactor A was limited by light supply and, therefore, microalgal activity. The pH of the recycling cultivation broth remained approximately constant at ≈7.8, which confirms the robust microbial activity in this enclosed photobioreactor configuration (Muñoz et al., 2009). The periodic monitoring of CH_4 and CO_2 concentrations in the enclosed headspace of the settling chamber of the tubular photobioreactor during stage III, revealed that while the recorded CH_4 content (<1.6%) indicated a negligible anaerobic TOC biodegradation, the CO_2 percentage (<8.6%) confirmed the occurrence of aerobic organic matter oxidation. On the other hand, TOC and IC removals of $81 \pm 7\%$ and $94 \pm 6\%$, respectively, were recorded during stage II in the open biofilm photobioreactor, while these REs accounted for $78 \pm 59\%$ and $98 \pm 1\%$ during stage III. The low pH values recorded in this particular biofilm photobioreactor in stages II and III (Table 3) promoted by the high nitrification activity of the open algal–bacterial biofilm ($52 \pm 5\%$ and $46 \pm 3\%$ of the inlet N-NH_4^+, respectively) caused an intense CO_2 removal by stripping since only $44 \pm 29\%$ and $13 \pm 4\%$ of the total carbon removed was recovered in the harvested biomass in stages II and III, respectively. In this context, the estimated CO_2 concentrations in the bulk cultivation medium (0.6 ± 0.1 mg L^{-1}) in stages II and III were higher than aqueous CO_2 concentration (0.3 mg L^{-1}) in equilibrium with its atmospheric concentration. The carbon assimilated as nitrifying biomass only represented $3.2 \pm 0.6\%$ and $3.2 \pm 0.4\%$ of the inlet IC in stages II and III, respectively.

In the tubular photobioreactor, biomass accumulation inside the tubes, caused by the high adherence of the biomass onto the tubes, induced the gradual decrease in nutrient removals recorded during stages II and III. Thus, TN-REs in photobioreactor A decreased to $48 \pm 17\%$ and $33 \pm 11\%$ during stage II and III, respectively. Likewise, the low DOs imposed by the higher organic loading rates resulted in a low nitrifying activity (<5% in both stages) and in a gradual decrease in N-NH_4^+-RE to $63 \pm 9\%$ and $13 \pm 4\%$ in stages II and III, respectively (Fig. 3b). Likewise, P-PO_4^{3-}-REs in photobioreactor A decreased to negative values during stages II and III likely due to the hydrolysis of the biomass accumulated inside the tubes and to the fact that non-structural phosphorous is released from microalgae under O_2 deprived conditions (Alcántara et al., 2013). On the other hand, a decrease in nutrient removal efficiency caused by the increased C losses by stripping (which hindered nutrient removal by assimilation) was also recorded in the photobioreactor B. In this

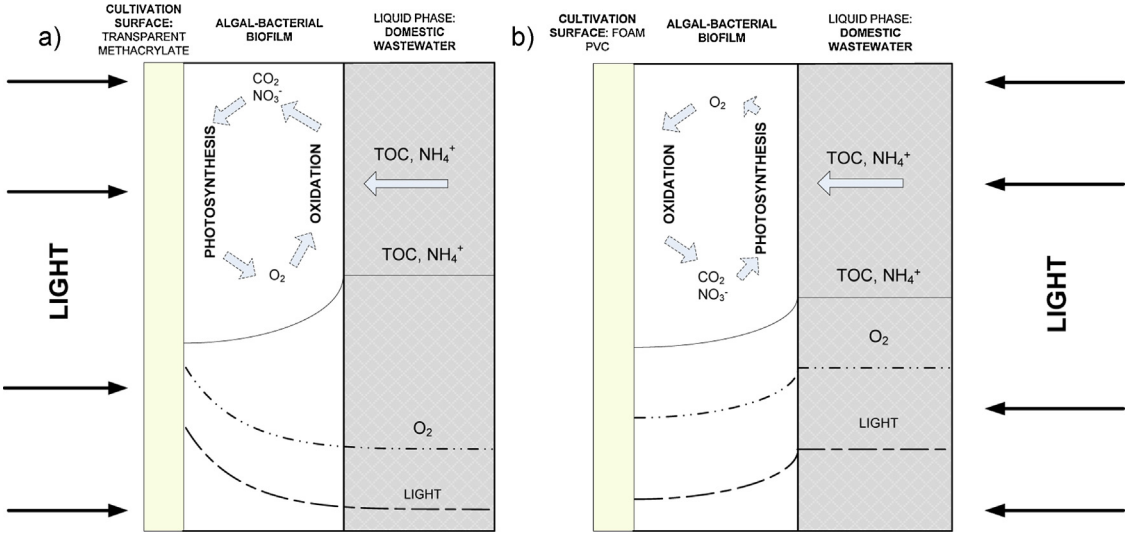

Fig. 6. Proposed mechanism for TOC and NH_4^+ oxidation within the biofilm attached onto the enclosed tubular photobioreactor wall (a) and onto the open biofilm photobioreactor surface (b).

context, TN-RE decreased to 48 ± 8% and 28 ± 1% in stages II and III, respectively. Surprisingly, despite the low pH values recorded in the open biofilm photobioreactor (6.7–7.3), nitrogen was mainly removed by stripping since only 38.0 ± 20.1% and 20.9 ± 6.0% of the TN removed in stages II and III, respectively, was recovered in the harvested biomass. However, N-NH_4^+ was completely removed at HRTs of 7 and 5 days. The contribution of nitrification to this NH_4^+ removal in stages II and III accounted for 52 ± 5% and 46 ± 3%, respectively, which supported maximum N-NO_3^- effluent concentrations of 81 mg N-NO_3^- L^{-1} during stage II. In our particular case, the carbon limitation encountered in the cultivation medium likely hindered N-NO_3^- assimilation into biomass both from a carbon and energy availability viewpoint. P-PO_4^{3-}-REs during photobioreactor B operation in stages II and III accounted for 49 ± 15% and 27 ± 18% (5 ± 2 and 4 ± 3 mg L^{-1}), respectively, with biomass assimilation being the main removal mechanism (76 ± 18% and 90 ± 20% of the total P removed was recovered in the harvested biomass, respectively).

The TSS-REs recorded in the effluent of both photobioreactors during stages II and III were comparable to those recorded in stage I due to the high adherence of the algal–bacterial biofilm onto the photobioreactor walls. Thus, a negligible biomass was harvested in photobioreactor A during stages II and III (≤0.003 g m^{-2} d^{-1}), while the algal–bacterial biomass harvested in photobioreactor B during both periods accounted for 3.6 ± 0.8 and 0.63 ± 0.1 g m^{-2} d^{-1} (PE of 2.8 ± 0.4% and 0.5 ± 0.1%, respectively). The above discussed deterioration in biomass productivity and nutrient removal recorded in stage III, concomitant with the increase in loading rates, was mediated by the carbon limitation imposed in the open photobioreactor by IC stripping.

Water evaporation losses in photobioreactor B promoted the deterioration of effluent quality. Thus, despite positive TN and PO_4^{3-} REs were always recorded, the high average water evaporation rates (≈3.7 L m^{-2} d^{-1}) entailed sometimes higher concentrations of TN and PO_4^{3-} in the effluent (104 and 15 mg L^{-1}, respectively) than in the influent (96 and 10 mg L^{-1}, respectively) at stages II and III. Therefore, the high water footprint together with the intense C stripping could eventually jeopardize the technical and environmental sustainability of open biofilm photobioreactors at large scale (Posadas et al., 2013).

A detailed analysis of the influence of the HRT on the RE for each target parameter in both photobioreactors clearly showed that higher HRTs supported similar or higher REs, except for IC-RE in the open photobioreactor due to the concomitant decrease in pH (associated to nitrification), which favored IC stripping. The recorded TOC-REs corresponded to the maximum TOC biodegradable fraction of the wastewater and were not significantly affected by the HRT in the tested interval. Based on data extrapolation, the optimum HRT for complete IC and TN removal in the enclosed system would be 11.9 and 12.1 d, respectively, while complete removals of TN and P in the open would require HRTs of 10.6 and 10.4 d, respectively (Fig. S1 in Supplementary Material).

3.2. Influence of the effluent recycling rate and pH on the performance of wastewater treatment

Stages IV (increase in effluent recycling from 4.2 to 9 L m^{-2} min^{-1}) and V (increase in the cultivation pH to 9.5) were conducted in order to promote biofilm detachment from the tubular photobioreactor and prevent inorganic carbon stripping in the open system, respectively.

The high adherence of the algal–bacterial biofilm to the tubes hindered biomass detachment (Chai and Zhao, 2012) and consequently, the nutrient removal capacity gradually deteriorated in the tubular photobioreactor as a consequence of an excessive biomass accumulation. The increase in effluent recycling flow from 27 to 60 cm s^{-1} caused no significant biofilm detachment despite the increase in shear stress over the biofilm, as shown by the negligible biomass harvesting in the settling chamber (Fig. 5). In this regard, the high adherence of the algal–bacterial biomass onto the tubes could limit the technical viability of this technology in the long term operation if no innovative biofilm detachment strategy is developed. Likewise, a higher recirculation rate did not entail a higher biomass detachment from photobioreactor B. Finally, the increase in liquid recycling did not result in an enhanced carbon and nutrient removal in the photobioreactor configuration evaluated or in a different contribution of the removal mechanisms to their fate.

The sudden pH increase in the cultivation broth (stage V) of both photobioreactors caused a severe damage in the microalgal–bacterial community of the biofilms and the development of a new algal–bacterial population adapted to the new operational conditions. A significant biomass detachment occurred in both photobioreactors as a result of the likely death of the microbial population, as shown by the increase in the effluent TSS concentration and biomass harvested (Fig. 5). In the tubular algal–bacterial biofilm photobioreactor, the maximum solid concentration recorded during stage V in the effluent was 0.14 g TSS L^{-1} and up to 1.48 g biomass m^{-2} d^{-1} (compared to 0.01 g m^{-2} d^{-1} in stage IV) were harvested. Photobioreactor A did not acclimate to the new operating conditions and underwent a rapid deterioration in TOC, IC and TN removals. Thus, a TOC-RE of 42% along with a negative IC removal were recorded at the end of stage V. Likewise, the gradual hydrolysis of the biomass accumulated into the tubes and the slow acclimation of the new algal–bacterial community likely supported negative TN and P removals. On the other hand, the rapid biomass detachment following the step pH increase in photobioreactor B resulted in effluent solid concentrations of 0.18 g TSS L^{-1} by day 130 (≈negligible TSS removal). However, the system rapidly acclimated to the new operational conditions with TOC-REs of 47% after 8 d. The operation of the open algal–bacterial photobioreactor at pHs >9 resulted in a complete recovery of the C removed in the harvested biomass, which was supported by the estimated CO_2 concentration in the bulk liquid phase (0.1 mg L^{-1}). This performance also resulted in an increase in P-PO_4^{3-} REs to ≈50%. Finally, despite TN-REs accounted for ≈100%, only 17% of the TN removed was recovered in the harvested biomass, NH_3 stripping being the main N removal mechanism.

3.3. Carbon, nitrogen and phosphorus biomass composition

The C, N and P content of the algal–bacterial biomass harvested remained constant regardless of the photobioreactor tested and operational conditions. The C and N content of the harvested biomass in photobioreactor A represented 43.1 ± 2.3% and 7.6 ± 0.7% on a total dry weight basis (Table 4). The phosphorus content (0.9%) was determined only in stage V due to the lack of harvested biomass in the previous stages. Likewise, the C, N and P content of the harvested biomass in photobioreactor B accounted for 44.8 ± 1.5%, 8.5 ± 0.6% and 1.0 ± 0.2%. These biomass compositions were similar to the C, N and P content reported by Dominguez Cabanelas et al. (2013) (43–56%, 2–9% and 1–4%, respectively) in algal biomass by treating domestic wastewater.

3.4. Microalgae population

The microalgae inoculum was composed of (percentage of cells) *Woronichinia* sp. (59.2%), *Acutodesmus* sp. (36.4%), *Aulacoseira* sp. (1.5%), *Desmodesmus quadricaudatus* (1.3%), *Nitzschia* sp. (1.0%), *Limnothrix redekei* (0.5%) and *Gomphonema parvulum* (0.1%).

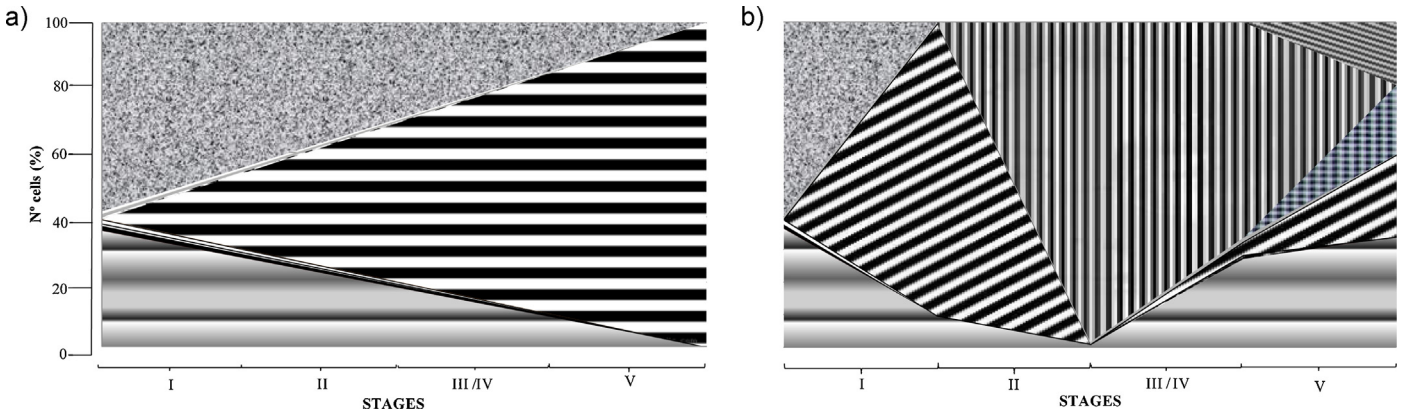

Fig. 7. Time course of microalgae population in photobioreactor A (a) and B (b). *Acutodesmus* sp., *Aulacoseira* sp., *Chlorella* sp., *Desmodesmus quadricaudatus*, *Leptolyngbya foveolara*, *Limnothrix redekei*, *Nitzschia* sp., *Planktothrix* cf. *prolifica*, *Pseudanabaena limnetica*, *Synechocystis aquatilis and* *Woronichinia* sp.

This share dramatically changed when considering microalgae densities, which ranged from 0.003 cells μm^{-3} in *G. parvulum* to 35.3 cells μm^{-3} in *L. redekei* (Table 1, supplementary materials). The configuration of biofilm photobioreactor did influence the final microalgae population, with a significantly larger diversity recorded in the open system (Fig. 7).

The characterization of microalgae population in photobioreactor A was carried out only for stage V due to the absence of algal–bacterial biomass harvested along stages I, II, III and IV (Fig. 7a). This entailed a linear microalgae population dynamic, which was likely to occur throughout the entire operational period due to the fact that enclosed photobioreactors offer a more protected environment to the microalgae than their open counterparts (Zittelli et al., 2004). Surprisingly, none of the main microalgae species initially present in the inoculum survived at the end of the experiment. Thus, *Leptolyngbya foveolara* (*Phormidium foveolarum*) was the dominant microalgae identified in photobioreactor A at the end of the stage V. The genus *Phormidium* was ranked 12 in the ranking of most tolerant microalgae in HRAPs devoted to wastewater treatment (Palmer, 1969). The higher microalgae biodiversity found in photobioreactor B might be explained by the higher risk of contamination of open cultures (Zittelli et al., 2004). *Acutodesmus* (a former subgenus within *Scenedesmus*) and *Woronichinia* sp. gradually disappeared during stage I (Fig. 7b), *L. redekei* (*Oscillatoria redekei*) being identified as the predominant specie (90.1%) in stage I and *Pseudanabaena limnetica* (*Oscillatoria limnetica*) (98.5%) in stage II. *Oscillatoria* and *Scenedesmus* were ranked in the top 6 of most pollution tolerant microalgae in HRAPs (Palmer, 1969). Despite *P. limnetica* continued being dominant during stage III (66.3%), *Acutodesmus* sp. increased up to 27.2%. *Nitzchia*, *Chlorella* and *L. redekei* (*O. redekei*) were detected during stage III at 1.1%, 1.3% and 4.1%, respectively. The genera *Chlorella* and *Nitzchia* were also ranked among the most pollution tolerant microalgae in HRAPs (Palmer, 1969). Stage IV entailed an increase in *Acutodesmus* concomitant with the decrease in the population of *P. limnetica*. Finally, stage V supported the highest diversity among the operational periods evaluated, likely promoted by the higher C availability mediated by the prevention of IC stripping: *Acutodesmus* (34.0%), *L. redekei* (25.4%), *Synechocystis aqualis* (19.3%) and *Planktohrix* cf. *profilica* (1.3%). Similar microalgal genera were identified in outdoors HRAPs treating diluted pig slurry (Fallowfield and Garret, 1985; De Godos et al., 2009a; Groeneweg et al., 1980) and domestic wastewater (García et al., 2000).

In brief, despite the current research was conducted indoors, the results here obtained confirmed the difficulty to maintain monoalgal cultures in open photobioreactors due to the variability in wastewater composition and environmental conditions, and to the complex interactions between microalgae and bacteria within biofilms (De Godos et al., 2009a).

Table 4
Carbon, nitrogen and phosphorus content of the harvested algal–bacterial biomass on a dry weight basis in both photobioreactors under the different operational stages evaluated.

Photobioreactor	Stage	Carbon (%)	Nitrogen (%)	Phosphorus (%)
A	I	N.D.	N.D.	N.D.
B		46.4	8.9	0.8
A	II	44.8	7.6	N.D.
B		43.9	8.6	1.2
A	III	43.6	6.9	N.D.
B		45.4	8.7	1.1
A	IV	39.8	7.2	N.D.
B		N.D.	N.D.	N.D.
A	V	44.4	8.6	0.9
B		42.5	7.1	1.1

N.D., non-determined due to the insufficient biomass harvested.

4. Conclusions

This study confirmed the higher carbon removal capacity of open biofilm photobioreactors as a result of the significant contribution of stripping to carbon removal. Similar nitrogen removals were achieved in enclosed and open systems due to either limitations of light supply promoted by the high biomass adherence onto the tubes in the enclosed system or carbon limitations in the open system. Phosphorus was only removed efficiently in the open photobioreactor. Despite the enclosed photobioreactor prevented abiotic carbon and nitrogen losses, its performance at low HRTs must be further optimized to the consistent levels achieved in the open photobioreactor. Finally, a constant biomass composition was recorded during the performance in both photobioreactors, with the open photobioreactor supporting a higher microalgal biodiversity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2014.03.007.

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Glossary

C: carbon
C_{in}*:* influent concentration
C_{out}*:* effluent concentration
DO: dissolved oxygen concentration
E: specific heating value of the dry microalgal biomass
G: impinging irradiation
HRAP: high rate algal pond
HRT: hydraulic retention time
IC: inorganic carbon
N: nitrogen
P: phosphorus
PAR: photosynthetically active radiation
PE: photosynthetic efficiency
Q_{in}*:* influent flowrate
Q_{out}*:* effluent flowrate
RE: removal efficiency
TN: total nitrogen
TOC: total organic carbon
TSS: total suspended solids
VSS: volatile suspended solids
W: areal microalgal–bacterial biomass productivity

SUPPLEMENTARY MATERIALS

Table 1. Microalgae/cyanobacteria population in the inoculum.

Microoalgae/cyanobacteria	Cell density (unit µm^{-3})	N° cells (%)	Volume (%)
Acutudesmus	5.0	36.4	6.2
Aulacoseira sp.	0.2	1.5	9.2
Desmodesmus quadricaudatus	0.2	1.3	8.5
Gomphonema parvulum	0.003	0.1	30.8
Limnothrix redekei	35.3	0.5	15.1
Nitzschia	0.03	1.0	21.2
Woronichinia sp.	5.0	59.2	9.0

Table 2. Microalgae/cyanobacteria population in photobioreactor A during stage V.

Microoalgae/cyanobacteria	Cell density (unit µm^{-3})	N °cells (%)	Volume (%)
Leptolyngbya foveolara (Phormidium fovelarum)	25.0	100	100

Table 3. Microalgae/cyanobacteria population in photobioreactor B during stage I.

Microoalgae/cyanobacteria	Cells density (unit µm^{-3})	Nº cells (%)	Volume (%)
Acutudesmus	1.3	9.7	53.2
Limnothrix redekei	100.0	90.1	3.9
Nitzschia	0.03	0.2	43.0

Table 4. Microalgae/cyanobacteria population in photobioreactor B during stage II.

Microoalgae/cyanobacteria	Cells density (unit µm^{-3})	Nº cells (%)	Volume (%)
Acutudesmus	0.05	1.0	40.6
Nitzschia	0.02	0.6	55.7
Pseudanabaena limnetica (Lemmermann)	50.0	98.5	3.71

Table 5. Microalgae/cyanobacteria population in photobioreactor B during stage III.

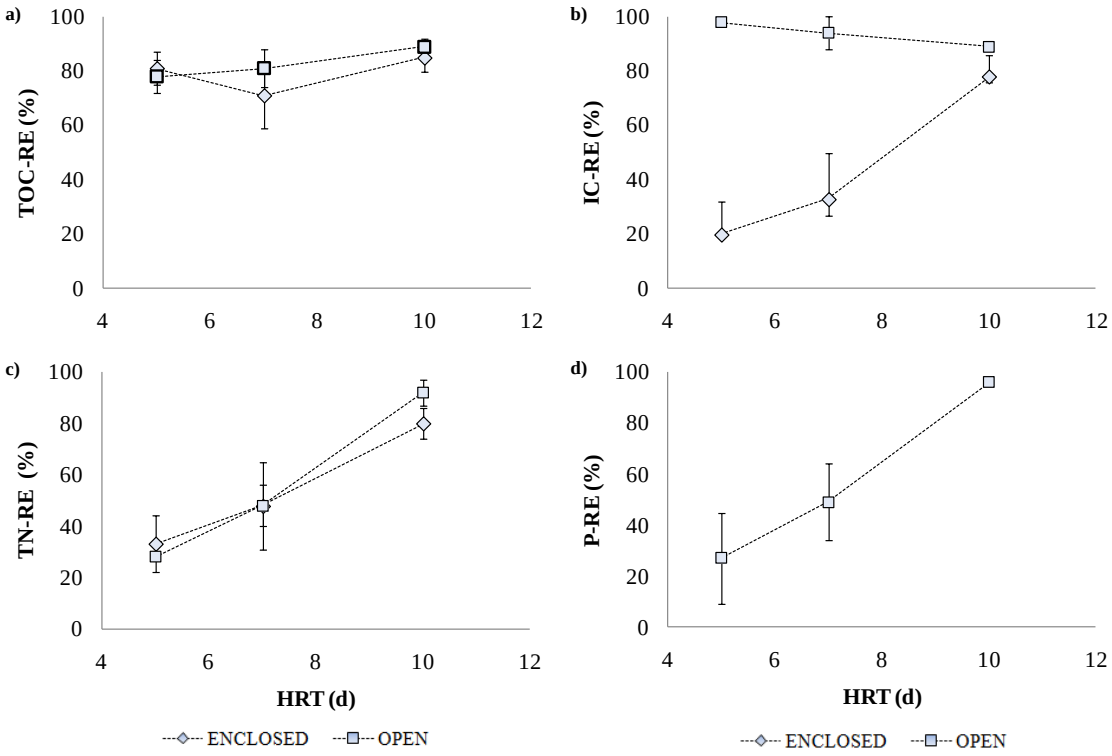
Microoalgae/cyanobacteria	Cells density (unit μm^{-3})	Nº cells (%)	Volume (%)
Acutudesmus	2.5	27.2	15.9
Chlorella sp.	0.1	1.3	30.5
Limnothrix redekei	2.5	4.1	2.8
Nitzschia	0.04	1.1	49.8
Pseudanabaena limnetica (Lemmermann)	100.0	66.3	0.9

Table 6. Microalgae/cyanobacteria population in photobioreactor B during stage V.

Microoalgae/cyanobacteria	Cells density (unit μm^{-3})	Nº cells (%)	Volume (%)
Acutudesmus	1.7	34.0	49.8
Limnothrix redekei	10.0	25.4	6.2
Planktothrix cf. prolifica (Gomont)	1.3	21.3	31.5
Synechocystis aquatilis Sauvageau	10.0	19.3	4.5

Figure S1.

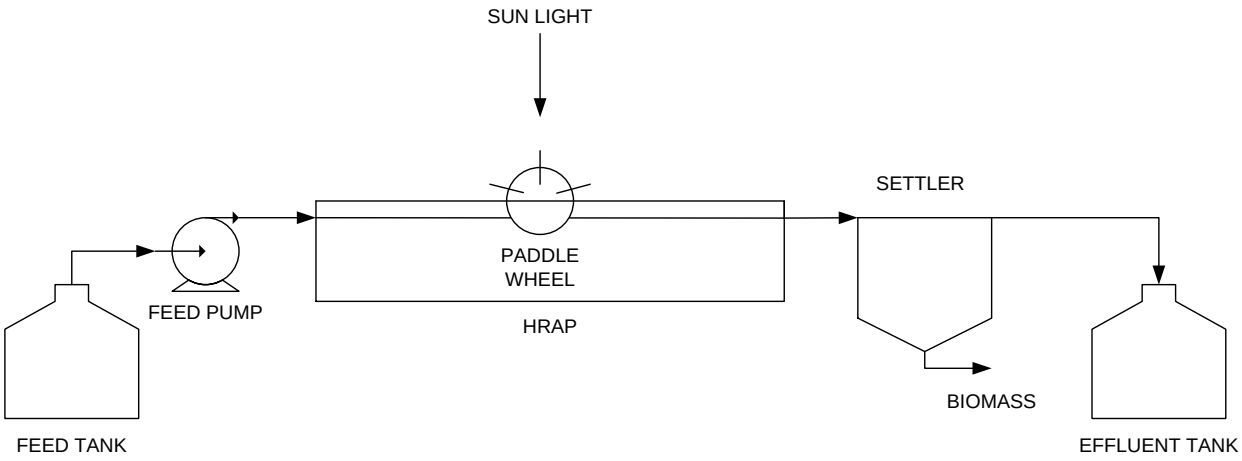
Influence of the HRT on the RE (%) of **a)** TOC, **b)** IC, **c)** TN and **d)** P in the enclosed and open photobioreactors.



A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters

Posadas E., Muñoz A., García-González M.-C., Muñoz R., García-Encina P. A. (2015), J. Chem. Technol. Biotechnol. 90 (6): 1094-1101.

Chapter 6



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A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters

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ABSTRACT

BACKGROUND: Microalgae-based technologies have emerged as a promising approach for simultaneous wastewater treatment and fish food production able to overcome the main limitations in the aquaculture sector. The current study focused on the mechanisms of carbon and nutrient removal from fish farm and domestic wastewaters in a 180 L outdoors pilot high rate algal pond (HRAP) at different hydraulic retention times (HRTs) considering water evaporation losses.

RESULTS: Maximum chemical oxygen demand, total Kjeldahl nitrogen and total phosphorus removal efficiencies of 77 ± 9%, 83 ± 10% and 94 ± 6%, respectively, were recorded during the treatment of fish farm wastewater at 10 d of HRT. Carbon and nitrogen were removed by assimilation into biomass (52 ± 12% and 74 ± 22%, respectively) and stripping, while phosphorus was mainly assimilated into biomass (69 ± 23%). Carbon stripping, along with the low carbon and nutrient loading rates supplied, resulted in low biomass productivities (maximum of 5 g m^{-2} d^{-1}). A successful solids removal was achieved in the settler (82 ± 18%), which entailed effluent solid concentrations below the maximum permissible discharge limit.

CONCLUSION: Despite the successful wastewater treatment supported by the HRAP, the high water evaporation losses (up of 15 L m^{-2} d^{-1}) could compromise the technical and environmental viability of this green wastewater treatment technology.

Keywords: algae; bacteria; bioreactors; biotreatment; environmental biotechnology; wastewater treatment and waste minimisation

NOTATION

AB	Percentage of C, N and P removed by assimilation into biomass
C_{in}	Influent concentration (mg L^{-1})
C_{out}	Effluent concentration (mg L^{-1})
COD	Chemical oxygen demand (mg L^{-1})
DO	Dissolved Oxygen concentration (mg L^{-1})
E	Specific heating value of the dry microalgal biomass (MJ kg^{-1})
G	Impinging irradiation (MJ $m^{-2}_{\text{surface photobioreactor}}$ d^{-1})
HRT	Hydraulic retention time (d)
IC	Inorganic carbon (mg L^{-1})
PAR	Photosynthetically active radiation (μmol m^{-2} s^{-1})
PE	Photosynthetic efficiency (%)
Q_{in}	Influent flow rate (L d^{-1})
Q_{out}	Effluent flow rate (L d^{-1})
RE	Removal efficiency (%)
TKN	Total Kjeldahl nitrogen (mg L^{-1})
TN	Total nitrogen (mg L^{-1})
TOC	Total organic carbon (mg L^{-1})
TP	Total phosphorus (mg L^{-1})
TSS	Total suspended solids (g L^{-1})
VSS	Volatile suspended solids (g L^{-1})
W	Areal microalgal-bacterial biomass productivity (kg m^{-2} d^{-1})

INTRODUCTION

Today, the agroindustry is the main industrial sector in Spain with 20% and 17% of the total national industrial production and employment, respectively.[1,2] Consequently, large amounts of waste are generated in this activity. The total waste produced by the Spanish agroindustry in 2010 was more than 3 million tons.[3] Agroindustrial wastewaters, which constitute a significant fraction of these wastes, are highly seasonal and dependent on the specific raw material being processed.[4] Despite their variable flow rates and concentrations, agroindustrial wastewaters are characterized by high organic matter and nutrient concentrations.[5] The uncontrolled disposal of such effluents into the environment results in water pollution and eutrophication of rivers and lakes.[6] Stricter wastewater discharge regulations have been enforced, which could compromise the economic viability of the agroindustrial

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sector.[7] Therefore, the development of cost-effective, environmentally friendly and sustainable technologies for the treatment of agroindustrial effluents is necessary.

Aquaculture has emerged as the fastest growing food production sector worldwide, with an annual average growth rate of 7.1% in 2010.[8] Spanish fish production constitutes 10% of the total European production, only exceeded by Norway.[8] However, this rapid growth is nowadays challenged by the development of cost-effective water management and fish feeding strategies, since 40% of the global aquaculture production depends on commercial fed stocks.[9] In this regard, microalgae-based processes have emerged as a promising alternative for the simultaneous treatment of wastewater and fish food production (Fig. 1).

This sun-powered technology is characterized by the photosynthetic production of oxygen by microalgae, which is used *in situ* by bacteria to oxidize the organic matter present in the wastewater into the CO_2 required by microalgae. Nutrient removal takes place by assimilation into algal-bacterial biomass, which constitutes a valuable product for fish feeding.[10,11] Thus, microalgae-based wastewater treatment can support both free wastewater oxygenation and nutrient recovery in the form of fish feed, overcoming the main drawbacks of traditional wastewater treatment methods such as activated sludge and anaerobic processes.[12]

The simple and cost-effective implementation of this technology in high rate algal ponds (HRAPs), together with the need for a low maintenance and operational control, represent key advantages for the implementation of algal-bacterial processes in agro-companies.[13] Preliminary studies focused on coupling fish farm wastewater treatment with the production of algal biomass have been carried out over recent decades. For instance, de la Noüe *et al.*[14] and Sevrin-Reyssac [15] operated a HRAP treating fish farm wastewater mixed with swine manure effluent in order to cultivate algal biomass for *Daphnia* sp. feeding. However, based on the preliminary nature of these studies, the optimization of fish farm wastewater treatment in hatcheries is mandatory to avoid compromising the sustainability and economics of aquaculture.

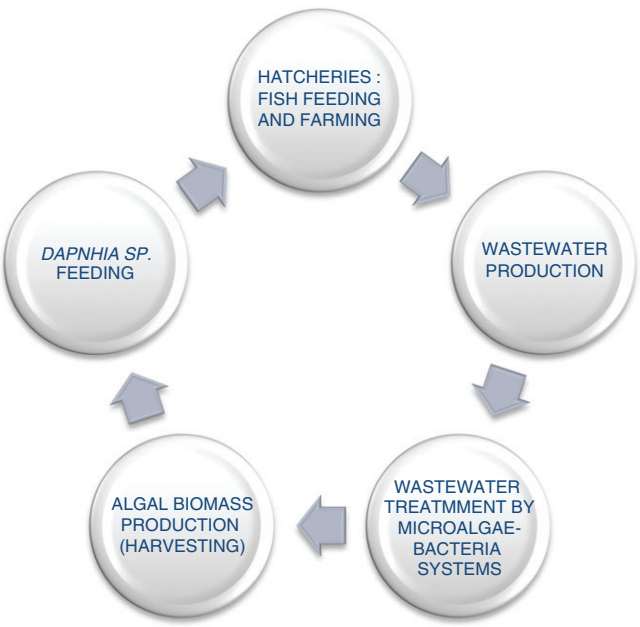

Figure 1. Integration of microalgae-based wastewater treatment in the aquaculture industry.

In this work, the ability of an outdoors pilot algal-bacterial HRAP for the removal of organic matter and nutrients from fish farm wastewater was evaluated for 6 months (April 2012–September 2012) under continental weather conditions at different loading rates. This research was devised to optimize the hydraulic residence time (HRT) and to identify both the main limitations during fish farm wastewater treatment in HRAPs and the potential of the co-degradation of this wastewater with domestic wastewater.

MATERIALS AND METHODS

Fish farm and domestic wastewaters

Fish farm wastewater was obtained from the Aquaculture Research Centre of the Institute of Agricultural Technology of Castilla y León (Segovia, Spain). Domestic wastewater was collected from a public sewer system nearby the Department of Chemical Engineering and Environmental Technology (University of Valladolid, Spain). These fresh wastewaters were pre-treated by primary sedimentation (3 h) and maintained at 4 °C while being pumped into the HRAP. The performance of the HRAP was subjected to daily variations in the composition of the received fish farm and domestic wastewaters (Table 1).

Experimental set-up

The pilot plant was located outdoors on the roof of the Department of Chemical Engineering and Environmental Technology at Valladolid University (41.39° N, 4.44° W). The experimental set-up consisted of a raceway constructed in flexible PVC with 180 L total working volume, 1.33 m^2 of illuminated surface (170 cm long, 82 cm wide, 15 cm deep) and two water channels divided by a central wall. Culture mixing was provided by a six bladed paddle wheel driven by a motor operated at 10.5 rpm (KELVIN K 200), which supported a liquid velocity of 22 cm s^{-1}. The sedimentation of the HRAP cultivation broth was carried out in an 8 L settler located at the outlet of the HRAP (Fig. 2). The HRAP was initially filled with 168 L of tap water and inoculated with a microalgae consortium (10 L at 0.7 g of TSS L^{-1}) collected from a HRAP treating diluted centrates at the Department of Chemical Engineering and Environmental Technology (University of Valladolid, Spain), and activated

Table 1. Composition of the fish farm and domestic wastewaters

	Wastewater	
Parameter	Fish farm	Domestic
Chemical oxygen demand (mg O_2 L^{-1})	678 ± 249	412 ± 119
Total organic carbon (mg C L^{-1})	161 ± 67	155 ± 66
Inorganic carbon (mg C L^{-1})	65 ± 37	100 ± 20
Total nitrogen (mg N L^{-1})	31 ± 10	92 ± 12
Total Kjeldahl nitrogen (mg N L^{-1})	33 ± 17	96 ± 8
NH_4^+ (mg N-NH_4^+ L^{-1})	10 ± 8	68 ± 17
Total phosphorus (mg P L^{-1})	19 ± 5	11 ± 3
PO_4^{3-} (mg P-PO_4^{3-} L^{-1})	14 ± 7	10 ± 3
Carbon:Nitrogen:Phosphorus*	100:14:6	100:36:4
Total suspended solids (g L^{-1})	0.2 ± 0.2	0.2 ± 0.1
pH	6.7 ± 0.4	7.5 ± 0.4

Neither NO_2^- nor NO_3^- were recorded in the initial characterization of the wastewaters.
*Ratio calculated from the TC (TOC + IC), TN and PO_4^{3-} concentrations.

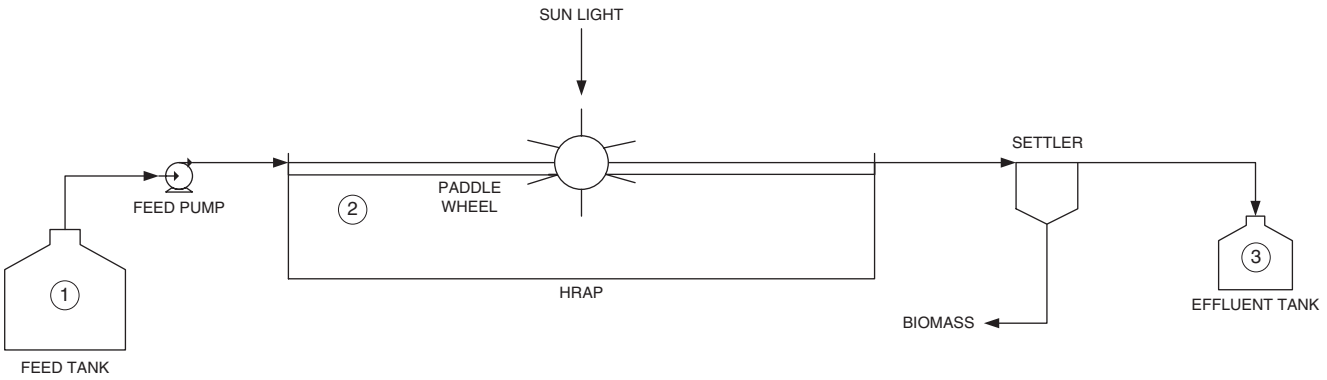

Figure 2. Experimental set-up of the outdoors HRAP. The numbers 1, 2, and 3 denote the sampling ports used for characterization of the influent, reactor and effluent, respectively.

Table 2. Operational conditions during the 153 days of the HRAP operation

N° stage	Time (d)	Wastewater	Feed flow ($L\,m^{-2}\,d^{-1}$)	HRT (d)	pH	COD ($g\,m^{-2}\,d^{-1}$)	C[a] ($g\,m^{-2}\,d^{-1}$)	N[b] ($g\,m^{-2}\,d^{-1}$)	P[c] ($g\,m^{-2}\,d^{-1}$)
I	1-20	F. F.	7.1	20	6.8 ± 0.2	3.9 ± 1.5	1.5 ± 0.5	0.2 ± 0.1	0.2 ± 0.1
II	21-69	F.F.	14.3	10	6.8 ± 0.3	10.2 ± 3.1	3.2 ± 2.0	0.5 ± 0.3	0.3 ± 0.1
III	70-100	F.F.	28.6	5	6.6 ± 0.6	20.5 ± 8.5	7.7 ± 1.8	1.1 ± 0.4	0.5 ± 0.3
IV	101-153	F.F. + D. (50:50)	19.5	7	7.3 ± 0.2	9.9 ± 4.5	3.6 ± 0.7	1.2 ± 0.3	0.2 ± 0.1

[a] Carbon load was calculated from TOC and IC concentrations;
[b] Nitrogen load was estimated from TKN concentrations;
[c] Phosphorus load was calculated from TP concentrations. F.F. = fish farm; D. = domestic.

sludge (2 L at 2.5 g TSS L^{-1}) obtained from Valladolid wastewater treatment plant (WWTP).

The HRAP was fed continuously with fish farm wastewater using a Watson Marlow 102 UR pump at HRTs of 20, 10 and 5 d during the first three stages and with a mixture of fish farm and domestic wastewaters (50%/50%) at 7 d in stage IV (Table 2). Domestic wastewater was supplemented during stage IV in order to balance the nitrogen concentration supplemented into the HRAP and, consequently, to enhance both fish wastewater treatment and microalgae growth in the HRAP. Except during the first start-up stage, the operational conditions were maintained in each stage until a steady state was reached. The total chemical oxygen demand (COD), carbon (C), nitrogen (N) and phosphorus (P) loading rates ranged from 3.9 ± 1.5 to 20.5 ± 8.5 g COD m^{-2} d^{-1}, 1.5 ± 0.5 to 7.7 ± 1.8 g C m^{-2} d^{-1}, 0.2 ± 0.1 to 1.2 ± 0.3 g TKN m^{-2} d^{-1} and 0.2 ± 0.1 to 0.5 ± 0.3 g TP m^{-2} d^{-}, respectively.

The temperature and dissolved O_2 concentration (DO) in the cultivation broth of the HRAP, and the influent and effluent (drawn from the outlet of the settler) flow rates were daily measured at 9 a.m. The daily average external temperature, precipitation and wind speed in Valladolid were obtained from the local meteorological station. The photosynthetic active radiation (PAR) was monitored weekly at 11:00 a.m., while the average sun irradiation (G) and number of hours of daylight were obtained from the database of the Photovoltaic Geographical Information System.[16] Liquid samples of 300 mL from the influent and effluent of the system were drawn twice a week to monitor the pH and the concentration of COD, total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), total Kjeldahl nitrogen (TKN), NH_4^+, NO_2^-, NO_3^-, total phosphorus (TP), PO_4^{3-}, total suspended solids (TSS) and volatile suspended solids (VSS). The determination of TOC, IC, TN, NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} concentrations was carried out only in the soluble phase before filtration through 0.20 μm nylon filters. Likewise, 100 mL of the HRAP cultivation broth were drawn twice a week to monitor the pH and the concentrations of TSS and VSS. Biomass composition (C, N, P content) was analyzed only during steady state operation. The percentage of impinging radiation stored as chemical energy into biomass, namely photosynthetic efficiency (PE), was estimated according to:

$$PE = \frac{W \cdot E}{G} \cdot 100 \quad (1)$$

where W represents the areal biomass productivity ($kg\,m^{-2}{}_{surface\ photobioreactor}\ d^{-1}$), E the specific heat value of dry microalgal biomass (22.5 MJ kg^{-1} according to USDOE[17]) and G the impinging solar irradiation ($MJ\,m^{-2}{}_{surface\ photobioreactor}\ d^{-1}$).

The COD, carbon, nitrogen and phosphorous removal efficiencies (RE) were calculated taking into account the water evaporation losses in the HRAP according to:

$$RE = \frac{\left(Q_{in} \cdot C_{in} - Q_{out} \cdot C_{out}\right)}{Q_{in} \cdot C_{in}} \cdot 100 \quad (2)$$

where Q_{in} represents the influent flow rate ($L\,d^{-1}$); Q_{out} the effluent flow rate ($L\,d^{-1}$) and C_{in} and C_{out} are the influent and effluent concentrations ($mg\,L^{-1}$) of the target monitored parameters, respectively.

The percentage of solids removed from the cultivation broth in the settler ($RE_{settler}$) was quantified according to:

$$RE_{settler} = \frac{TSS_{photobioreactor} - TSS_{effluent}}{TSS_{photobioreactor}} \cdot 100 \quad (3)$$

where $TSS_{photobioreactor}$ and $TSS_{effluent}$ correspond to the TSS concentration (g TSS L^{-1}) in the HRAP and in the effluent (after sedimentation), respectively.

Biomass harvesting was carried out once a week by wasting the settler supernatant and centrifuging the algal blanket of the settler at 10 000 rpm for 10 min at 23 °C (Sorvall/Legend RT, Thermo Scientific, USA). The areal biomass productivity (W) was determined according to Ozkan *et al.*[18]:

$$W = \frac{W_{net}}{S \cdot t} \quad (4)$$

where W_{net} is the harvested biomass (g) dried at 105 °C for 24 h in a P-Selecta laboratory stove (SELECTA, Spain); S the HRAP illuminated surface (1.33 m^2) and t is the elapsed time between harvesting (d). Equation (4) was validated based on the effluent flow rate, TSS concentration in the HRAP and $RE_{settler}$ according to:

$$W = \frac{TSS_{photobioreactor} \cdot Q_{out}}{S} \cdot \frac{RE_{settler}}{100} \quad (5)$$

The percentage of C, N and P removed by assimilation into biomass (AB) was calculated according to:

$$AB = \frac{\left(Q_{in} \cdot C_{in} - Q_{out} \cdot C_{out}\right) - W \cdot \%_{composition}}{\left(Q_{in} \cdot C_{in} - Q_{out} \cdot C_{out}\right)} \cdot 100 \quad (6)$$

where W is expressed in mg d^{-1} and $\%_{composition}$ corresponds to the composition of C, N and P in the biomass (%) of the harvested biomass.

Analytical procedures

The parameters COD, TKN, TP, TSS and VSS concentrations were analyzed according to APHA Standard Methods.[19] TOC, IC and TN concentrations were determined using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 Chemiluminescence module. N-NH_4^+ concentration was quantified using an Orion Dual Star ammonia electrode (Thermo Scientific, The Netherlands). N-NO_3^-, N-NO_2^- and P-PO_4^{3-} concentrations in the soluble phase were analyzed via HPLC-IC using a Waters 515 HPLC pump coupled with a conductivity detector (Waters 432) and equipped with an IC-PAK Anion HC column (4.6 × 150 mm) and an IC-Pak Anion Guard-Pak (Waters). A Eutech Cyberscan pH510 pHmeter (Eutech instruments, Spain) was used for pH determination. DO and water temperature were recorded using an OXI 330i oximeter (WTW, Germany). The PAR radiation was measured with a LI-250A light meter (LI-COR Biosciences, Germany). The determination of the C and N biomass content was performed using a LECO CHNS-932, while phosphorus biomass content was carried out spectrophotometrically after biomass digestion according to Standard Methods (Spectrophotometer U-2000, Hitachi, Japan).

RESULTS AND DISCUSSION

The average biomass concentration recorded in the cultivation broth over the 153 days of operation and the suspended solid removal efficiency in the settler accounted for 0.64 ± 0.61 g TSS L^{-1} and 82 ± 18%, respectively (Fig 3). The average biomass productivity in the HRAP was 2.1 ± 0.60 g m^{-2} d^{-1}, which represented a PE of 0.2%. These productivities were significantly lower than those reported by Hoffmann[20] (10–35 g m^{-2} d^{-1}) in an outdoors HRAP treating domestic wastewater at HRTs ranging from 2 to 6 d.

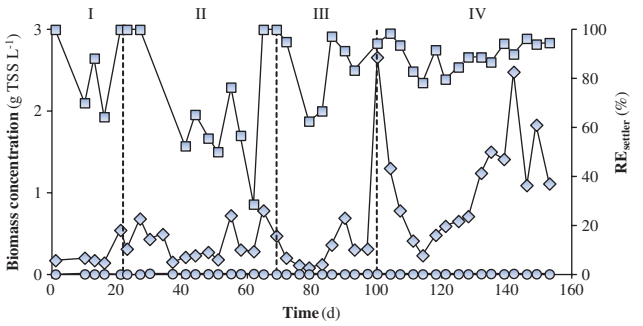

Figure 3. Time course of biomass concentration in the cultivation broth (diamonds) and in the effluent (circles), and biomass removal efficiency in the settler (squares) during treatment of the fish farm and domestic wastewaters.

The low C and nutrient loads supplied to the HRAP, together with active CO_2 and NH_4^+ stripping mediated by the high turbulence at this pilot scale, probably explain the low biomass productivities recorded (Table 2).

The composition of the harvested biomass remained constant regardless of the supplied load, with an average C, N and P content (on a dry weight basis) of 43 ± 3%, 7.1 ± 0.6% and 1.1 ± 0.3%, respectively. These values were within the typical range reported for algal-bacterial biomass.[21] On average, according to Equation (6) 52 ± 12%, 74 ± 22% and 69 ± 23% of the total carbon, nitrogen and phosphorus removed from the fish farm and domestic wastewaters in the HRAP, respectively, was recovered in the harvested biomass. Hence, assimilation into biomass and CO_2 stripping contributed equally to carbon removal (≈50%). In this context, the average estimated dissolved CO_2 concentration in the cultivation broth during the 153 operational days was 1 ± 0.7 mg L^{-1}, which was higher than the aqueous CO_2 equilibrium concentration with the atmospheric CO_2 (0.4 mg L^{-1}). Similarly, up to 59% of the influent carbon was removed by stripping in a 464 L HRAP treating 10 fold diluted swine slurry.[12] The high turbulence in the pilot scale HRAP as a result of the use of high power engines was probably responsible for the high contribution of CO_2 stripping to carbon removal in this particular study.

Similarly, nitrogen removal occurred via assimilation into biomass and NH_4^+ volatilization, despite the low share of dissolved NH_3 in the cultivation broth (0.7 ± 0.5% of the total NH_4^+ input). A ≈ 58% contribution of NH_3 volatilization was recorded in an open pond treating diluted swine manure at a pH of 8.5 and HRTs of 6.7–2.8 d at 37 °C.[22] De la Noüe *et al.*[14] recorded ammonia volatilization losses of 98% during winter (T < 5 °C, pH = 8–10) in an open raceway treating swine manure diluted up to 100-fold with fish pond effluent. Therefore, ammonia stripping was confirmed as one of the main mechanisms for nitrogen removal in open ponds as a result of the high pHs mediated by microalgae photosynthesis. Assimilation into biomass and precipitation at high pHs were hypothesized as the two major mechanisms for phosphorous removal.[23] In our particular case, 69 ± 23% of the P was removed by assimilation into biomass. Finally, the C/N/P ratio for fish farm and domestic wastewaters was 100/14/6 and 100/36/4 (Table 1), respectively. Based on the optimal reported ratio for microalgae growth of 100/18/2[24], the ratios recorded here suggest a potential nitrogen limitation in stages I, II and III and carbon limitation in stage IV during the co-treatment of fish farm and domestic wastewaters.

Table 3. Average values of temperature, DO and pH of the cultivation broth, ambient temperature, light intensity, daylight hours and evaporation losses in each operational stage

N°Stage	T_{HRAP} (°C)*	DO (mg O_2 L^{-1})*	pH_{HRAP}	T_{out} (°C)	Light intensity (Wh $m^{-2}d^{-1}$)	Light time (h)	Evaporation losses (L m^{-2} d^{-1})
I	9 ± 2	11 ± 1	8.6 ± 1.1	14 ± 5	5435	9 ± 1	3 ± 8
II	15 ± 6	9 ± 4	8.7 ± 0.4	19 ± 4	5892	11 ± 1	11 ± 3
III	19 ± 5	8 ± 4	8.3 ± 0.2	22 ± 3	5995	11 ± 1	12 ± 3
IV	13 ± 2	11 ± 3	8.7 ± 0.3	21 ± 4	4677	8 ± 1	13 ± 2

*Parameters measured every day at 9:00 a.m.

Stage I

The HRAP was initially fed with fish farm wastewater at 20 d of HRT in order to acclimate the algal-bacterial community, a slightly high value compared with the typical range of 3–15 d during steady wastewater treatment by algae.[25] Stage I supported the most unfavorable environmental conditions for the development of biological activity in the HRAP in terms of external temperatures, wind speed and rain (data not shown). The average solar irradiation and number of daylight hours were 5435 Wh m^{-2} d^{-1} and 9 ± 1 h, respectively, which are typical values in Castilla y León at the end of April (Table 3). Despite these harsh environmental conditions, high COD and TOC removal efficiencies of 77 ± 10% and 84 ± 7%, respectively, were recorded within this first period (Fig. 4(a) and 4(b)) probably due to the high DO prevailing in the cultivation broth during this stage (11 ± 1 mg O_2 L^{-1}). The biodegradability of fish farm wastewater was tested according to González *et al.*[26] and accounted for 75 ± 2% in terms of COD removal, which matched the COD removal recorded in the HRAP during this start-up period. IC-REs accounted for 57 ± 12% (Fig. 4(c)) and occurred via assimilation into algal biomass due to the lack of nitrifying activity and the fact that the estimated CO_2 concentration gradient in stage I promoted CO_2 absorption from the atmosphere rather than stripping.

The high level of treatment achieved during stage I for TKN-RE (91 ± 8%) (Fig. 5(a)) confirmed the high nitrogen removal capacity of algal-bacterial HRAPs.[12] Similarly, TN-RE accounted for 80 ± 16% (Fig. 5(b)) while NH_4^+ was almost completely depleted (98 ± 2%) (Fig. 5(c)). Despite the high DO and sufficient IC in the cultivation broth (36 ± 7 mg C L^{-1}), neither nitrite nor nitrate were recorded in stage I. In this regard, the nitrifying activity was likely limited by the low NH_4^+ concentrations mediated by NH_3 stripping and assimilation (Fig. 5(c)). On the other hand, the negligible contribution of nitrification to NH_4^+ removal could be explained by the low fraction of nitrifying bacteria present in the cultivation broth during process start-up.[27] Likewise, high TP and PO_4^{3-} REs of 84 ± 14% and 78 ± 17%, respectively, were recorded during stage I (Fig. 6).

Despite this successful carbon and nutrient removal, a negligible biomass productivity (0.5 ± 0.4 g m^{-2} d^{-1}) was achieved. The average suspended solids concentration in the cultivation broth during this start-up phase was 0.3 ± 0.2 g TSS L^{-1}, while no significant washout of biomass was noticed despite the rains. The suspended solid removal efficiency in the settler was 85 ± 13%.

Stage II

The HRAP was then challenged by feeding fish farm wastewater at 10 d of HRT from May till June 2012. Stage II was characterized by average irradiations of 5892 Wh m^{-2} d^{-1}, 11 ± 1 h of daylight and average external temperatures of 19 ± 4 °C, which mediated evaporation losses of 11 ± 3 L m^{-2} d^{-1}. These high evaporation rates were promoted by the high temperatures together with the high turbulence present in the HRAP as a result of the use of a high power engine and absence of vane smoothing the change in direction of the cultivation broth.[28] The HRAP evaporation rates were similar to those reported by the *Algal simulator*[29] program developed by Massey University (New Zealand) in a 0.3 m depth HRAP located in Yuma (Arizona) at 10 d of HRT in June (10 L m^{-2} d^{-1}). As a result of these water evaporation losses, a significant increase in the effluent concentrations of the different

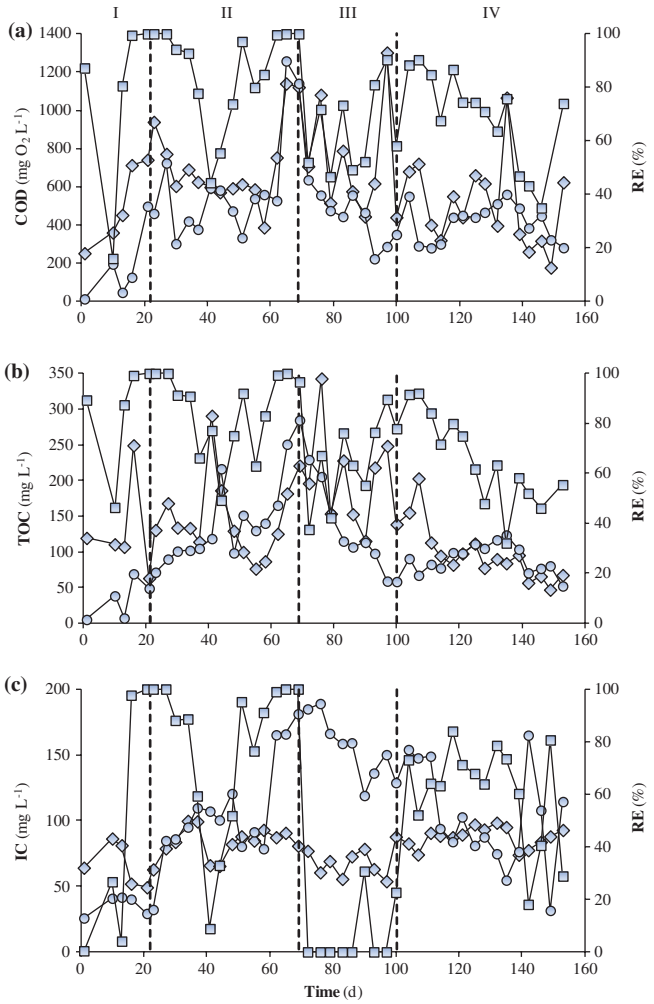

Figure 4. Time course of COD (a), TOC (b) and IC (c) concentrations in the influent (diamonds), effluent (circles) and removal efficiencies (squares) during the treatment of the fish farm and domestic wastewaters.

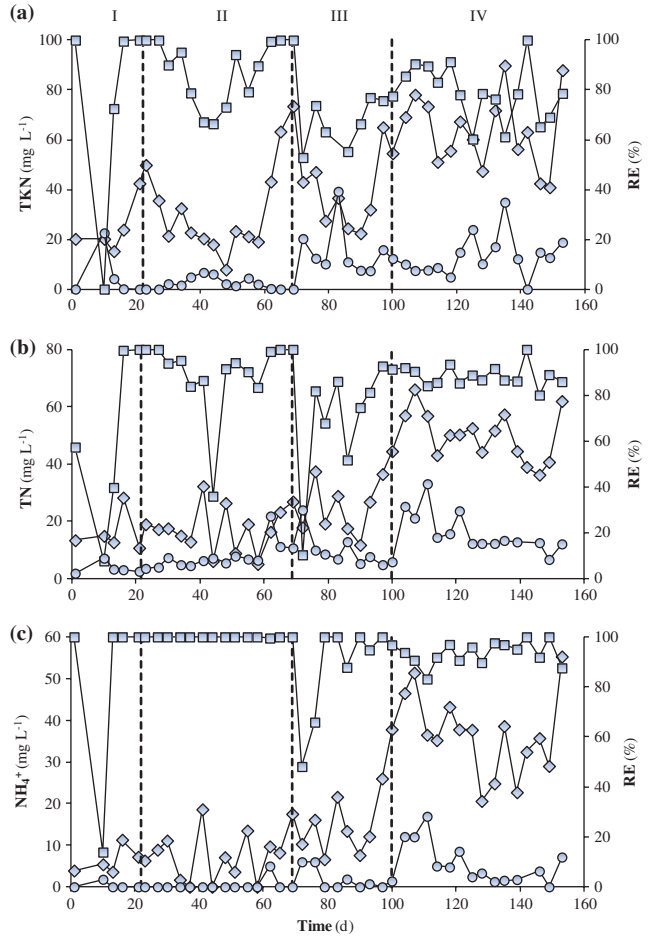

Figure 5. Time course of TKN (a), TN (b) and NH_4^+ (c) influent concentrations (diamonds), effluent concentrations (circles) and removal efficiencies (squares) during the treatment of the fish farm and domestic wastewaters.

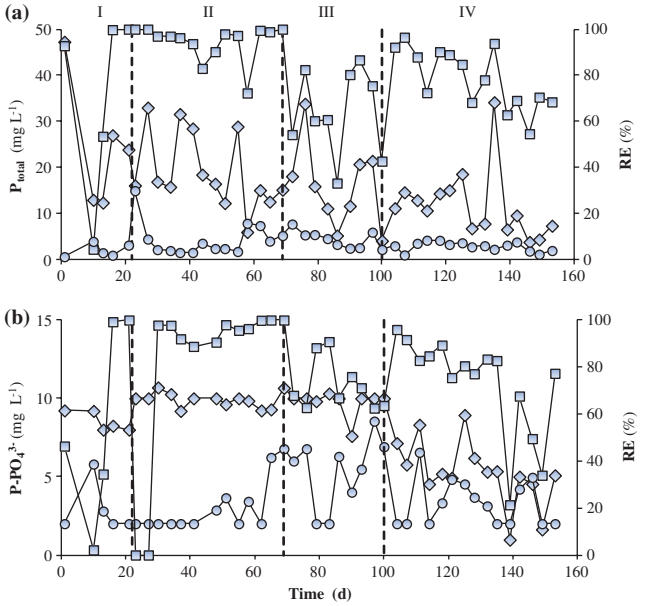

Figure 6. Time course of total phosphorus (a) and phosphate (b) influent concentrations (diamonds), effluent concentrations (circles) and removal efficiencies (squares) during the treatment of the fish farm and domestic wastewaters.

parameters monitored was noticed. For instance, despite the positive TOC mass removal achieved at steady state in stage II, the final average effluent TOC concentration (145 ± 64 mg L^{-1}) was similar (and sometimes higher) than the influent TOC concentration (148 ± 57 mg L^{-1}) (Fig. 4(b)). Indeed, when the RE of the target monitored parameters was 100% during stage II the effluent flow rate was negligible as a result of the high evaporation (Fig. 4; Fig. 5; Fig. 6). This high water footprint represents one of the main disadvantages of outdoors HRAPs, despite a recent life cycle analysis concluded that the yearly average evaporation rates could be negligible due to the impact of the rains in tropical countries.[30,31] The higher temperatures along with the increase in organic matter loading rate in stage II (Table 2), likely mediated the recorded DO decrease from 9.6 to 6.6 mg O_2 L^{-1}. A removal efficiency for COD, TOC and IC of $77 \pm 9\%$, $86 \pm 10\%$ and $86 \pm 12\%$, respectively, was achieved (Fig. 4). The estimated CO_2 concentration gradient under these particular operating conditions supports the occurrence of CO_2 stripping.

TKN, TN and NH_4^+-REs were $83 \pm 10\%$, $85 \pm 8\%$ and $100 \pm 0\%$, respectively (Fig. 5), while TP and PO_4^{3-} REs accounted for $94 \pm 6\%$ and $99 \pm 1\%$, respectively (Fig. 6). These nutrient removal efficiencies were in agreement with those reported by Groeneweg *et al.*[32] in a HRAP treating diluted pig manure at 10 d of HRT and pH of 9.6 (94% NH_4^+-RE and 93% P-RE, respectively).

A maximum biomass productivity of 1.1 g m^{-2} d^{-1} was recorded in stage II. This higher value compared with stage I was inherent to the higher C, N and P load supplied into the system. The highest biomass concentration in the HRAP was reached at the end of stage II (0.78 g TSS L^{-1}), while the lowest settler performance among the four stages evaluated was achieved in stage II (TSS-RE of $70 \pm 25\%$).

Stage III

The HRT was further decreased to 5 d under a fish farm wastewater feeding regime in order to increase HRAP productivity, during a period when the highest light irradiation and external temperatures (5995 Wh m^{-2} d^{-1} and 22 ± 3 °C, respectively) were recorded. The average water evaporation losses in stage III accounted for 12 ± 3 L m^{-2} d^{-1}, which also mediated a significant deterioration in the quality of the effluent. Although these favorable environmental conditions could have promoted higher microalgae activity, lower carbon and nutrient REs were recorded during stage III probably due to saturation of the bioremediation capacity of the HRAP. In this particular stage, the higher temperatures and organic matter loading rates also mediated a decrease in DO concentrations to 3.1 mg O_2 L^{-1}, but always above the inhibitory threshold of 2 mg O_2 L^{-1} for both organic matter and NH_4^+ oxidation.[27] Compared with stage II, COD and TOC-REs decreased to $64 \pm 16\%$ and $65 \pm 17\%$, respectively (Figs. 4(a) and 4(b)). Surprisingly, negative IC removal efficiencies were recorded despite the aqueous CO_2 concentration supporting significant IC stripping (Fig. 4(c)). This suggests that CO_2 from TOC oxidation was released at higher rates than IC assimilation into algal biomass and CO_2 stripping.

TKN, TN and NH_4^+ REs remained high at $68 \pm 10\%$, $78 \pm 14\%$ and $93 \pm 12\%$ (Fig. 5), while lower REs were recorded for TP and PO_4^{3-} (64 ± 19 and $72 \pm 13\%$, respectively) (Fig. 6). Average biomass productivities of 2 ± 1 g m^{-2} d^{-1} were recorded in stage III, where the high evaporation losses induce biomass concentrations in the cultivation broth of up to 2.7 g TSS L^{-1}. However, a successful suspended solid removal ($84 \pm 14\%$) was recorded in the settler.

Stage IV

The co-treatment of domestic wastewater with fish farm wastewater, which allowed balance of the nitrogen concentration supplemented into the system in order to minimize the impact of the previous nitrogen limitation recorded in the algal-bacterial HRAP, was evaluated at 7 d of HRT (August–September 2012). The selection of 7 d for HRT during the co-treatment of domestic wastewater and fish farm wastewater allowed process operation at similar nitrogen loads to those treated in stage III, while COD, carbon and phosphorus loads decreased by approximately 60% (Table 2). Under this particular scenario, COD, TOC, TP and PO_4^{3-} inlet concentrations decreased, while IC, TKN, TN and NH_4^+ inlet concentrations increased.

Wastewater treatment in stage IV was carried out at lower irradiations and daylight hours (4677 Wh m^{-2} d^{-1} and 8 ± 1 h, respectively) than in previous stages. A decrease in ambient temperature to 21 ± 4 °C and in the cultivation broth to 13 ± 2 °C were also recorded, despite stage IV exhibiting the highest average evaporation losses (13 ± 2 L m^{-2} d^{-1}). These lower temperatures, together with the lower organic matter loads, probably promoted increase in the DO to 11 ± 3 mg L^{-1}. Likewise, this increase in the DO concentration could have been mediated by a higher photosynthetic activity, as was shown by the pH increase to 8.7 ± 0.3. Despite the inherent influence of temperature on the performance of this outdoors HRAP (a likely underestimated performance during stage I), the narrow interval of average ambient temperatures (13–19 °C) (Table 3) during stages II to IV (the first period corresponded to the tailoring of the HRAP) supports the assumption that the HRT was the most significant parameter influencing the algal-bacterial performance.

COD-REs in stage IV increased to 70 ± 17%, while TOC and IC REs remained constant at 65 ± 18% and 54 ± 19%, respectively (Fig. 4). TKN, TN and NH_4^+-REs were 79 ± 12%, 88 ± 5% and 93 ± 5% (Fig. 5). The highest influent NH_4^+ concentration in stage IV, together with the highest pHs recorded in the cultivation broth, promoted NH_4^+ volatilization, which accounted for 73 ± 15% of the TN removed. Similarly, TP and PO_4^{3-}-REs were 79 ± 15% and 78 ± 14% (Fig. 6). The co-treatment of both wastewaters supported the maximum biomass productivities recorded over the entire experimental period, 5 g m^{-2} d^{-1}, and the highest average biomass concentrations in the cultivation broth (1 ± 0.6 g TSS l^{-1}) (Fig. 3). Under these particular conditions, biomass removal in the settler achieved a maximum value of 90 ± 15%. These results were in agreement with the research carried out by Park *et al.*[33] in an 8000 L HRAP, where biomass removal by gravity sedimentation was a function of the intrinsic settleability of the algae population and directly proportional to the biomass concentration in the pond.

Finally, considering the maximum permissible discharge concentrations for COD, TKN, TP and TSS into the environment in the European legislation (125, 15, 2 and 35 mg L^{-1}, respectively), the HRAP only supported a proficient treatment performance in terms of TSS (average TSS of 7 ± 6 mg L^{-1} over the entire experimentation period).[7] COD, TKN and TP average effluent concentrations of 135 ± 102, 9 ± 7 and 2 ± 1 mg L^{-1}, respectively, would have been recorded in the absence of water evaporation losses during the four operational stages.

CONCLUSIONS

Maximum organic matter and nutrient removals from fish farm wastewater were recorded in the HRAP at 10 d of HRT. Carbon and nitrogen were removed by assimilation into biomass and stripping, while the phosphorus removed was mainly assimilated. Carbon stripping (in the form of CO_2), along with the low carbon and nutrient loading rates applied, limited microalgae productivity in the HRAP. An efficient biomass recovery took place in the settler, resulting in TSS concentrations below maximum European discharge limits. The high water footprint mediated an effluent quality deterioration, which could eventually jeopardize the effectiveness of this technology. Therefore, further research should focus on minimizing water evaporation rates by optimizing the turbulence in the cultivation broth.

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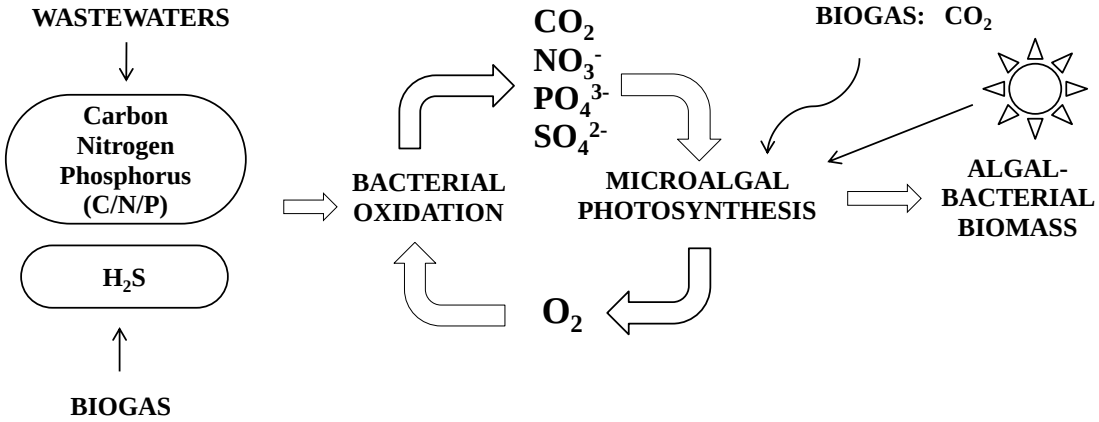
Algal-bacterial processes for simultaneous biogas upgrading and WWT

Chapter 7



Feasibility study of biogas upgrading coupled with nutrient removal from anaerobic effluents using microalgae-based processes

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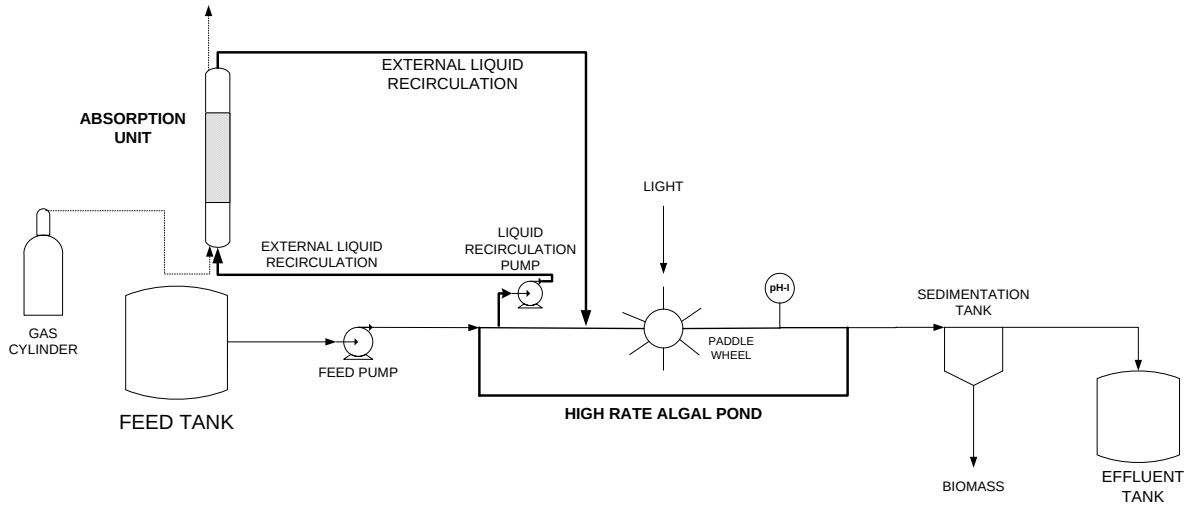
Chapter 7.1



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Feasibility study of biogas upgrading coupled with nutrient removal from anaerobic effluents using microalgae-based processes

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Abstract The present research was conducted to simultaneously optimize biogas upgrading and carbon and nutrient removal from centrates in a 180-L high-rate algal pond interconnected to an external CO_2 absorption unit. Different biogas and centrate supply strategies were assessed to increase biomass lipid content. Results showed 99 % CO_2 removal efficiencies from simulated biogas at liquid recirculation rates in the absorption column of 9.9 m^3 m^{-2} h^{-1}, concomitant with nitrogen and phosphorus removal efficiencies of 100 and 82 %, respectively, using a 1:70 diluted centrate at a hydraulic retention time of 7 days. The lipid content of the harvested algal–bacterial biomass remained low (2.9–11.2 %) regardless of the operational conditions, with no particular trend over time. The good settling characteristics of the algal–bacterial flocs resulted in harvesting efficiencies over 95 %, which represents a cost-effective alternative for algal biomass reutilization compared to conventional physical–chemical techniques. Finally, high microalgae biodiversity was found regardless of the operational conditions.

Keywords Algal–bacterial symbiosis · Biogas upgrading · Biological wastewater treatment · Microalgae lipid content · Microalgae population dynamics

Introduction

In the current global environmental and energy scenario, biogas upgrading coupled with nutrient removal from wastewaters in algal–bacterial photobioreactors constitutes a sustainable and promising alternative to conventional physical–chemical upgrading technologies (Bahr et al. 2014). This environmentally friendly technology, which also has low energy demands, is based on the solar-powered photosynthetic fixation of CO_2 from biogas by microalgae. The in situ-produced photosynthetic O_2 can be further utilized by sulfur-oxidizing bacteria to oxidize the H_2S absorbed from the biogas to SO_4^{2-} and by heterotrophic and nitrifying bacteria to oxidize the organic matter and nitrogen present in wastewaters to CO_2 and NO_3^-, respectively (Bahr et al. 2014; Muñoz and Guieysse 2006).

On the other hand, the current high price of fossil fuels together with their irreversible depletion and the accumulation of greenhouse gases derived from their combustion are currently promoting intensive research on the generation of biofuels from sustainable biomass sources (Chisti 2007; Christenson and Sims 2011). First- and second-generation biodiesel entails severe limitations, such as an extensive use of land (competing with human food crops) and costly

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pretreatments (Alam et al. 2012). In this context, biodiesel from microalgae constitutes a third-generation biofuel that is able to overcome the above-mentioned environmental and ethical limitations, based on the high productivity of microalgae cultures, the possibility of using low-quality water, and the lack of competition with crop land (Acién et al. 2012). Biodiesel from microalgae is obtained from the transesterification of their lipids, whose content can range from 5 to 77 % (on a dry weight basis) depending on the microalgae species and cultivation conditions (Chisti 2007).

The high current cost of monoalgal biomass cultivation (from 4.2 to 12.6 € kg^{-1} in 2010–2011) makes biodiesel from axenic microalgae non-competitive with conventional biodiesels, which requires a significant decrease in the biomass production costs (Acién et al. 2012; Norsker et al. 2011). The microalgae produced during the simultaneous upgrading of biogas and wastewater treatment constitute a low cost and sustainable biomass feedstock for biodiesel production because no CO_2 derived from fossil fuels is used (Acién et al. 2012; Bahr et al. 2014). High-rate algal ponds (HRAPs) represent the most versatile and cost-effective platform for the simultaneous treatment of wastewater and upgrading of biogas (Bahr et al. 2014; Park et al. 2011a, b). Unfortunately, this open photobioreactor configuration often undergoes contamination by native algae or zooplankton when sensitive microalgae are cultivated (De Godos et al. 2009; García et al. 2000; Park et al. 2011a, b). Therefore, the year-round predominance of lipid-rich microalgae in HRAPs seems unlikely based on the limited microalgae growth rates under lipid accumulation conditions (Breuer et al. 2012). In this context, nutrient deprivation has been shown as one of the most efficient strategies to induce storage lipid accumulation (Devi et al. 2013).

Despite the recent interest and intensive research conducted worldwide on microalgae cultivation as a promising technology for biofuel production, CO_2 mitigation, and wastewater treatment, the number of experimental studies evaluating the performance of such an integrated process is small (Park et al. 2011a, b; Serejo et al. 2015; Posadas et al. 2015). Thus, the main emphasis has been on pilot-scale experiments focusing on biogas upgrading and wastewater treatment (Bahr et al. 2014; Heubeck et al. 2007). Serejo et al. (2015) also evaluated the influence of the operational conditions on the chemical composition of the harvested biomass during the simultaneous biogas upgrading and wastewater treatment. In this study, we used a pilot-scale HRAP treating diluted wastewater centrate (the liquid fraction from the centrifugation of sludge digestate in a sewage treatment plant) and CO_2 from biogas. CO_2 uptake was optimized under nutrient deprivation to enhance microalgae lipid accumulation. In addition, a morphological characterization of the microalgae assemblages was conducted.

Materials and methods

Microorganisms and culture conditions. The HRAP was initially filled with 0.75 g total suspended solids (TSS) L^{-1} of a consortium of microalgae/cyanobacteria (henceforth referred to as microalgae) and bacteria treating diluted centrates (1:7) in a similar HRAP. The microalgae inoculum composition was as follows (% of cells): *Microspora* sp. (53.5 %), *Scenedesmus* (27.8 %), *Synechocystis aquatilis* (13.9 %), and *Woronichinia* sp. (4.7 %). This microalgae population was selected based on its previous acclimation to the characteristics of the diluted wastewater. The population composition changed significantly over time (Tables S1–S7 Supplementary material).

Simulated biogas and centrate. The simulated biogas used was composed of CO_2 (30 %) and N_2 (70 %) instead of CH_4 to avoid any explosion hazards (Abello Linde, Spain). H_2S was not included because its complete removal was reported by Serejo et al. (2015) regardless of the operational conditions. Centrate wastewater, which is characterized by a low biodegradable fraction and a high concentration of nutrients (Bahr et al. 2014; Posadas et al. 2013), was obtained from the digested sludge-concentrating centrifuges at Valladolid wastewater treatment plant (WWTP) (Spain) and stored at 4 °C prior to use. The centrate was diluted with tap water prior to feeding the HRAP to avoid microalgae inhibition because of its high NH_4^+ concentrations and to control the nutrient supply while compensating for water evaporation losses (Table 1) (González et al. 2008). In a real-world scenario, the use of the HRAP effluent (depleted of nutrients) as dilution water would significantly increase the economic and environmental sustainability of the process.

Experimental setup. The experimental setup consisted of a 15 cm deep 180-L HRAP, with an illuminated surface of 1.33 m^2 (202 cm length×63 cm width) and two water channels divided by a central wall, interconnected to a 2.5-L (Ø= 4 cm; height=195 cm) external CO_2 absorption column (CO_2-AC) via microalgae broth external recirculation (Fig. 1). The CO_2 absorption unit consisted of a bubble column with a ceramic sparger located at its bottom. The system was operated indoors at the Department of Chemical Engineering and Environmental Technology (University of Valladolid, Spain) for 225 days at an average temperature of 23±2 °C. The HRAP cultivation broth was continuously mixed by a six-blade paddlewheel at an internal recirculation velocity of approximately 20 cm s^{-1}. The surface of the HRAP was continuously illuminated at 75±5 μmol photons m^{-2} s^{-1} of average irradiation using 15 Gro-Lux fluorescent lamps (Sylvania, Germany). Despite Shriwastav and Bose (2015) reporting that intermittent illumination of 12-h light and dark periods at a light intensity of 246 μmol photons m^{-2} s^{-1} (≈light saturation

Table 1 Physical/chemical characteristics of the centrate used throughout the experimentation

Centrate	TOC (mg L^{-1})	IC (mg L^{-1})	TN (mg L^{-1})	N-NH_4^+ (mg N L^{-1})	N-NO_3^- (mg N L^{-1})	P_s (mg P L^{-1})	C/N/P ratio	TSS (g L^{-1})	pH
Raw	180±57	1390±382	681±122	446±101	10±1	72±28	100:43:05	0.03±0.00	7.9±0.3
Dilution 1:30	5.8±2.0	47.3±2.0	22.3±4.6	15.4±3.6	0.6±0.5	2.7±1.7	100:42:05	0.01±0.00	7.6±0.3
Dilution 1:70	2.8±0.6	27.8±0.2	10.2±0.9	6.0±1.1	0.5±0.4	1.4±1.0	100:33:05	0.01±0.00	7.5±0.3

NO_2^- was not detected

threshold for most microalgae species) was the optimal strategy for sustainable algal growth indoors, the continuous irradiation used in this particular research likely counterbalanced the low light irradiations used. Likewise, the successful results reported by Serejo et al. (2015) in a similar experimental setup and at slightly higher light irradiances (104±25 μmol photons m^{-2} s^{-1}) and light/dark cycles of 16:8 h showed sufficient microalgae–bacteria activity at the above-mentioned irradiation. Therefore, the low and continuous irradiances used here did not invalidate the main outcomes and experimental findings of this study. Effluent sedimentation was carried out in an 8-L settler operated at 9±1 h of hydraulic retention time (HRT) that was located at the outlet of the HRAP (Bahr et al. 2014).

Operational conditions, sampling procedure, and calculations. Six different operational conditions were tested to simultaneously optimize both CO_2 and nutrient removal from the simulated biogas and the diluted centrates, respectively, and the lipid content of microalgae. Based on these varied objectives, the achievement of an optimum microalgae growth along with the maximization of CO_2 removal in the AC and of lipid accumulation in the algal biomass was difficult. Therefore, the operational conditions were selected to obtain a balance for the target objectives. The HRT in the HRAP was maintained constant at 7 days during the entire experimental period, which corresponds to the optimum range for wastewater treatment in HRAPs (3–8 days) (Arbid et al. 2013; Posadas et al. 2015), while simulated biogas was sparged at 38.7±1.0 L day^{-1} concurrently with an external recycling of algal cultivation broth drawn from the HRAP (at 1.1, 3.5, or 9.9 m^3 m^{-2} h^{-1}or at their corresponding flow rate expressed in L day^{-1} of 34.8, 112.2, or 321.2, depending on the operational stage) (Fig. 1). The simulated biogas flow rate was chosen based on the results reported by Bahr et al. (2014) during the treatment of centrates diluted seven times at 23 days of HRT in a similar experimental setup. Centrate dilution was maintained at 1:30 from stages I to IV and increased to 1:70 in stages V and VI to limit nutrient supply to the HRAP and to promote lipid accumulation because nitrogen starvation has been reported as a successful strategy for increasing the lipid content in microalgae cells (Toledo-Cervantes et al. 2013). The areal carbon, nitrogen, and phosphorus loads

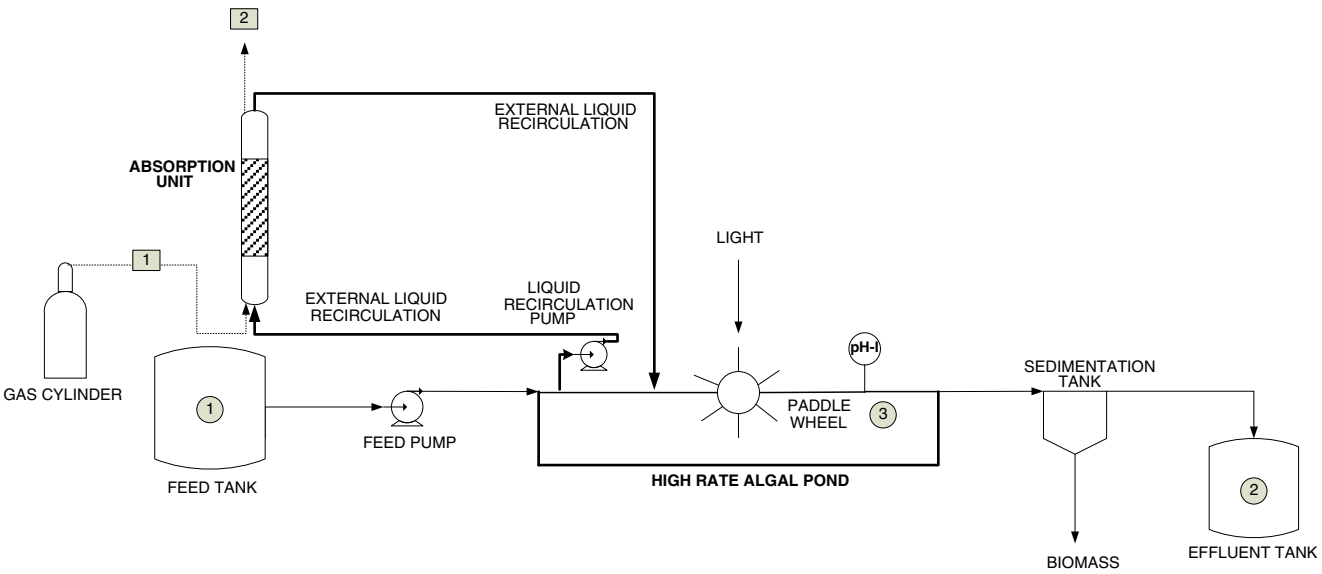

Fig. 1 Schematic diagram of the continuous biogas upgrading and nutrient removal experimental setup. *Grey circles* represent liquid sampling ports (*1*: influent, *2*: effluent; *3*: cultivation broth), and *grey squares* represent gas sampling ports (*1*: inlet biogas, *2*: upgraded biogas). *Continuous lines* represent liquid streams, and *dashed lines* represent biogas streams

supplied with the wastewater (and with the simulated biogas) ranged from 5.2 to 5.9 g C m^{-2} day^{-1}, 0.2 to 0.6 g N m^{-2} day^{-1}, and 0.02 to 0.05 g P m^{-2} day^{-1}, respectively (Table 2). The pH of the cultivation broth, the diffuser pore size in the absorption column, and the external liquid recirculation flow rate were modified throughout the different operational stages to increase the CO_2 mass transfer from the gas to the cultivation broth and, consequently, the overall C/N/P ratio available for microalgae growth and lipid accumulation (Park et al. 2011a, b). The pH of the cultivation broth was increased from 8.5 to 9.5 in stage II via automatic NaOH addition at 2 % *v*/*v* (Table 2). Similarly, the pore diameter of the diffuser was decreased from 10 μm (porous glass diffuser) to 2 μm (metallic diffuser) in stage III. On the other hand, the external recirculation flow rate between the CO_2-AC and the HRAP was maintained at 1.1 m^3 m^{-2} h^{-1} in the first three stages and increased to 3.5 m^3 m^{-2} h^{-1} in stages IV and V and to 9.9 m^3 m^{-2} h^{-1} in stage VI, which resulted in an increase in the liquid/gas (L/G) recirculation ratio from 0.9 to 2.9 and 8.3, respectively (Table 2). All of the tested conditions were maintained until a steady state was reached, which corresponded to ≈4–5 times the elapsed HRT.

Gas sampling (100 μL) was performed twice a week at the inlet and outlet of the CO_2-AC to monitor CO_2, O_2, and N_2 concentrations (Fig. 1). Similarly, the inlet and outlet gas flow rates were also measured to accurately determine CO_2 removal. Liquid sampling was also carried out twice a week by drawing 300 mL from the wastewater influent and effluent in the HRAP (Fig. 1) to monitor the concentrations of total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), NH_4^+, NO_2^-, NO_3^-, soluble phosphorus (P_s), TSS, volatile suspended solids (VSS), and pH. Likewise, liquid samples of 300 mL were drawn from the cultivation broth twice a week to monitor IC, TSS, and VSS concentrations. TOC, IC, TN, NH_4^+, NO_2^-, NO_3^-, and P_s concentrations corresponded to the soluble phase and required liquid sample filtration through 0.20-μm nylon filters prior to analysis.

The parameters measured daily were ambient and cultivation broth temperatures, dissolved O_2 (DO) concentration in the cultivation broth, and the influent and effluent wastewater flow rates, while light intensity at the HRAP surface was monitored weekly. Biomass harvesting was performed every 10 days by centrifugation of the biomass that had settled at the bottom of the sedimentation tank (Fig. 1).

The removal efficiency (RE) of CO_2 in the AC from the simulated biogas was quantified as follows:

$$\mathrm{RE}_{\mathrm{CO_2}} = \frac{C_{\mathrm{CO_2,IN}} \cdot \mathrm{F_{IN}} - C_{\mathrm{CO_2,OUT}} \cdot \mathrm{F_{OUT}}}{C_{\mathrm{CO_2,IN}} \cdot \mathrm{F_{IN}}} \cdot 100 \quad (1)$$

where $C_{CO2,IN}$ and $C_{CO2,OUT}$ are the concentrations (%) of CO_2 in the inlet and outlet biogas, respectively, in the CO_2-AC, while F_{IN} and F_{OUT} represent the inlet and outlet biogas flow rates (L day^{-1}), respectively. Likewise, the overall carbon RE was determined according to Eq. (2):

$$\mathrm{RE_C} = \frac{\left(C_{\mathrm{IN}} \cdot Q_{\mathrm{IN}} + C_{\mathrm{C\text{-}CO_2,IN}} \cdot F_{\mathrm{IN}}\right) - \left(C_{\mathrm{OUT}} \cdot Q_{\mathrm{OUT}} + C_{\mathrm{C\text{-}CO_2,OUT}} \cdot F_{\mathrm{OUT}}\right)}{\left(C_{\mathrm{IN}} \cdot Q_{\mathrm{IN}} + C_{\mathrm{C\text{-}CO_2,IN}} \cdot F_{\mathrm{IN}}\right)} \cdot 100 \quad (2)$$

where C_{IN} and C_{OUT} are the concentrations of total dissolved C (TOC + IC) in the influent and effluent wastewater (mg L^{-1}), respectively, and Q_{IN} and Q_{OUT} are the influent and effluent wastewater flow rates in the HRAP (L day^{-1}). In this particular calculation, $C_{C\text{-}CO2,IN}$ and $C_{C\text{-}CO2,OUT}$ are expressed in mg C-CO_2 L^{-1}.

Table 2 Operational conditions during the performance evaluation of the HRAP coupled with the external biogas upgrading column

	Absorption column			High-rate algal pond				
Stage	Liquid recirculation (m^3 m^{-2} h^{-1})	L/G ratio	Pore size $Ø_{diffuser}$ (μm)	Centrate (dilution)	pH	C[a] (g m^{-2} day^{-1})	N[b] (g m^{-2} day^{-1})	P[c] (g m^{-2} day^{-1})
I	1.1	0.9	10	1:30	8.4±0.2	5.9±0.2	0.4±0.1	0.04±0.02
II	1.1	0.9	10	1:30	9.5±0.1	5.4±0.3	0.4±0.1	0.04±0.02
III	1.1	0.9	2	1:30	9.4±0.2	5.6±0.3	0.6±0.1	0.05±0.03
IV	3.5	2.9	2	1:30	9.4±0.1	5.3±0.1	0.5±0.1	0.05±0.01
V	3.5	2.9	2	1:70	9.4±0.1	5.2±0.1	0.2±0.0	0.02±0.00
VI	9.9	8.3	2	1:70	9.4±0.1	5.2±0.1	0.2±0.0	0.02±0.00

[a] Total carbon (TC) loads were calculated considering both the influent TOC and IC concentrations and the C-CO_2 in the biogas flow rate

[b] Nitrogen loads were determined from the influent TN concentrations

[c] Phosphorus loads corresponded to soluble phosphorous concentrations based on the low TSS of the raw centrate (<30 mg TSS L^{-1})

TOC, IC, TN, and P_s REs were also estimated as follows:

$$RE_i = \frac{C_{i,IN} \cdot Q_{IN} - C_{i,OUT} \cdot Q_{OUT}}{C_{i,IN} \cdot Q_{IN}} \cdot 100 \quad (3)$$

where $C_{i,IN}$ and $C_{i,OUT}$ correspond, respectively, to the influent and effluent concentrations (mg L^{-1}) of the target monitored parameter i (TOC, IC, TN, or P_s).

The suspended solid RE of the settler ($RE_{settler}$) was calculated using Eq. (4):

$$RE_{settler} = \frac{TSS_{HRAP} - TSS_{effluent}}{TSS_{HRAP}} \cdot 100 \quad (4)$$

where TSS_{HRAP} and $TSS_{effluent}$ are the TSS concentrations (g TSS L^{-1}) in the HRAP and in the effluent, respectively. In this context, biomass productivity (W, g $m^{-2}{}_{surface\ HRAP} day^{-1}$) was quantified according to Eq. (5):

$$W = \frac{TSS_{HRAP} \cdot Q_{out}}{S} \quad (5)$$

where S represents the total HRAP illuminated surface.

Analytical procedures The gas (CO_2, N_2, and O_2) concentrations were determined using a Varian CP-3800 gas chromatograph (USA) equipped with a thermal conductivity detector and CP-Molsieve 5A (15 m×0.53 mm×15 μm) and CP-Pora BOND Q (25 m×0.53 mm×15 μm) columns. The concentrations of dissolved TOC, IC, and TN were measured using a Shimadzu TOC-VCSH analyzer (Japan) coupled with a TNM-1 chemiluminescence module. N-NH_4^+ concentration was determined with an NH_3 specific electrode, Orion Dual Star (Thermo Scientific, The Netherlands). The concentrations of N-NO_3^-, N-NO_2^-, and P-PO_4^{3-} were quantified by HPLC-IC as in De Godos et al. (2009). The concentration of soluble phosphorus was determined spectrophotometrically using the ammonium molybdate method. All analyses, including TSS and VSS, were conducted based on Standard Methods (Eaton et al. 2005). The pH of the algal broth was analyzed on-line using an R305 Consort system (Belgium), while a Eutech Cyberscan pH 510 (Eutech Instruments, The Netherlands) was used for pH determination in the HRAP influent and effluent. Temperature and dissolved oxygen concentration were determined using an OXI 330i oximeter (WTW, Germany). The photosynthetic active radiation (PAR) was measured with a LI-250A light meter (LI-COR Biosciences, Germany).

The algal–bacterial biomass harvested in the settler was dried for 24 h at 105 °C prior to characterization. The lipid content of the algal–bacterial biomass was determined gravimetrically following biomass extraction with a chloroform/methanol (2:1 *v*/*v*) solution (Kochert 1978). The determination of the C and N content of the algal–bacterial biomass was conducted in a LECO CHNS-932 analyzer, while the phosphorus content was determined spectrophotometrically after acid digestion in a microwave, according to Standard Methods (Eaton et al. 2005) based on the internal procedure of the Instrumental Technical Laboratory of Valladolid University. The identification, quantification, and biometry measurements of the microalgae population were performed by microscopic examination (Olympus IX70, USA) of biomass samples (fixed with Lugol's acid at 5 % and stored at 4 °C prior to analysis), as in Sournia (1978).

Results

Temperature, evaporation losses, and DO concentrations in the cultivation broth of the HRAP remained constant during the six operational stages (21–22 °C, 4–6 L m^{-2} day^{-1}, and 6–8 mg O_2 L^{-1}, respectively) (Table 3).

Stage I involved the start-up of the HRAP and was characterized by an RE_{CO2} of 47±9 % (Fig. 2).

Negligible RE_{TOC} values were recorded in the HRAP both in stage I and in the rest of operational stages evaluated, despite effluent TOC concentrations remaining low (ranging from 14 mg C L^{-1} in stage I to 4 mg C L^{-1} in stage V) (Table 3). The RE_{IC} and RE_C achieved during stage I (54±6 and 44±7 %, respectively) represented the highest REs during the 225 days of operation and represented the lowest IC concentration (47±6 mg C L^{-1}) in the cultivation broth. On the other hand, the RE_{TN} and RE_{Ps} were 99±1 and 74±7 %, respectively, resulting in negligible nitrogen concentrations (0.3 ±0.3 mg L^{-1}) in the HRAP and in P-PO_4^{3-} effluent concentrations of 1.7±0.4 mg L^{-1}. N-NH_4^+ was completely removed both in stage I and the rest of the operational stages (Table 3).

The lowest biomass productivity, TSS concentration in the culture broth, and $RE_{settler}$ (1.3±0.2 g m^{-2} day^{-1}, 0.11± 0.06 g L^{-1}, and 66.4±4.0 %, respectively) were recorded during this start-up period. The C, N, and P contents of the algal–bacterial biomass in stage I were 43.5, 7.9, and 1.0 %, respectively (Table 4).

The predominant *Microspora* sp. present in the inoculum was gradually replaced by *Pseudanabaena minima* during stage I, which also coexisted with *Scenedesmus* and *Limnothrix mirabilis*, each representing 15 % of the total population (Fig. 3).

The lipid content of the harvested biomass in stage I decreased from 7.4±2.1 % (in the inoculum) to 4.6±2.8 % (Table 4).

Once nitrogen was depleted in the cultivation broth in steady state I, the pH of the cultivation broth was increased from 8.5 to 9.5 to enhance CO_2 transfer from the biogas to the liquid phase in stage II, which resulted in similar RE_{CO2} to stage I (54±5 %). Negative RE_{IC} values were recorded from stage II onward, which led to a significant IC concentration

Table 3 Steady-state cultivation broth dissolved oxygen concentration and temperature and evaporation losses in the HRAP; removal efficiencies; effluent dissolved carbon (TC, TOC, and IC), nitrogen (TN, NH_4^+, and NO_3^-), and phosphorus (P_s) concentrations; and total suspended solid concentrations in the HRAP cultivation broth during the different stages

Parameters	Stage					
	I	II	III	IV	V	VI
DO (mg L^{-1})	8±1	7±1	6±0	6±0	6±0	7±0
T_{HRAP} (°C)	22±2	21±2	21±1	22±1	22±2	22±1
Evaporation losses (L m^{-2} day^{-1})	4±2	4±1	6±1	6±2	6±1	6±1
RE_C (%)	44±7	25±7	20±4	19±3	22±1	27±6
TOC effluent concentration (mg L^{-1})	14±1	6±1	8±1	9±1	4±1	7±1
IC effluent concentration (mg L^{-1})	47±6	114±14	117±4	212±15	211±6	228±15
RE_{TN} (%)	99±1	66±7	40±2	57±3	70±6	100±0
TN effluent concentration (mg L^{-1})	0.3±0.3	7±2	21±2	13±1	5±0	0
N-NH_4^+ effluent concentration (mg L^{-1})	0	0	0	0	0	0
N-NO_3^- effluent concentration (mg L^{-1})	0.3±0.3	7±2	17±1	12±1	4±0	0
RE_{Ps} (%)	74±7	48±5	51±5	45±10	53±12	82±3
P_s effluent concentration (mg L^{-1})	1.7±0.4	1.4±0.3	2.0±0.2	1.7±0.2	0.7±0.1	0.2±0.0
$RE_{settler}$ (%)	66.4±4.0	98.2±1.2	100.0±0.0	95.5±1.5	100.0±0.0	97.0±0.0
TSS cultivation broth (g L^{-1})	0.11±0.06	0.39±0.01	0.25±0.06	0.22±0.05	0.29±0.03	0.23±0.02

TC removal efficiency was calculated considering the TOC and IC removed from the wastewater and the C-CO_2 transferred from the biogas to the liquid phase

increase in the growth medium to 144 mg L^{-1} in stage II (Fig. 2). Similarly, RE_C also decreased during stage II to 25 ±7 % (Table 4). On the other hand, the RE_{TN} during stage II accounted for 66±7 %, with TN effluent concentrations of 7± 2 mg TN L^{-1} (which corresponded to nitrate concentrations). RE_{Ps} in stage II decreased to 48±5 %, with an effluent concentration of 1.4±0.3 mg L^{-1} (Table 4). Biomass productivity and suspended solid concentration in the HRAP increased to 5.4±0.2 g m^{-2} day^{-1} and 0.39±0.10 g TSS L^{-1}, respectively, while $RE_{settler}$ was 98.2±1.2 %. C, N, and P biomass contents in stage II were 35.6, 5.9, and 1.6 %, respectively. During stage II, *Chroococcidiopsis* sp. was the predominant microalgae in the HRAP, while *Microspora* sp. was again identified at abundances similar to those of *Synechocystis aquatilis* (11.2 and 9.4 %, respectively). The lipid content of the algal–bacterial biomass in stage II (5.2±2.1 %) was similar to that in stage I.

A similar RE_{CO2} (48±4 %) to previous stages was recorded when decreasing the diffuser pore size in stage III to increase the mass transfer area, which was expected to bring about an enhanced RE_{CO2} based on Fick's equation $D = K_a\ a\ \Delta C$ (Bird et al. 2006). The RE_C in this stage was 20±4 %, while the IC concentration in the HRAP remained similar to the previous stage. The RE_{TN} decreased to 40±2 %, which

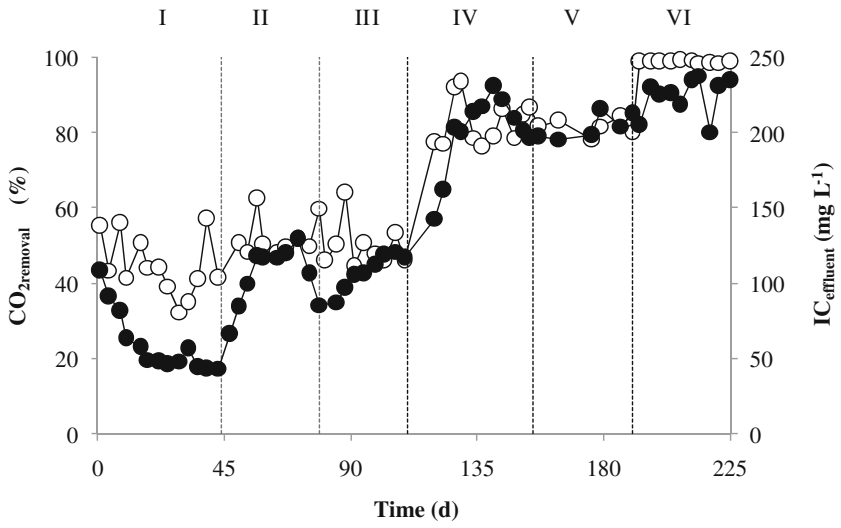

Fig. 2 CO_2 removal in the absorption column (*open circle*) and IC concentration in the cultivation broth (*close circle*) as a function of time

Table 4 Carbon, nitrogen, and phosphorus biomass contents and lipid composition in the inoculum and during the different stages

Parameters	Stage						
	Inoculum	I	II	III	IV	V	VI
Carbon (%)	41.4	43.5	35.6	38.5	40.1	42.5	43.6
Nitrogen (%)	6.3	7.9	5.9	6.5	7.1	6.2	7.5
Phosphorus (%)	0.8	1.0	1.6	1.4	1.3	1.6	1.4
Lipid (%)	7.4±2.1	4.6±2.8	5.2±2.1	8.9±3.2	4.1±1.0	7.4±0.4	3.3±0.7

represented the highest TN concentrations in the HRAP broth (21±2 mg L^{-1}), while RE_{Ps} remained constant at 51±5 %, resulting in a P_s concentration of 2 mg L^{-1}. Biomass productivity and concentration in the algal pond decreased in stage III to 3.9±0.9 g m^{-2} day^{-1} and 0.25±0.06 g L^{-1}, respectively, while a 100 % $RE_{settler}$ was recorded (Table 3). The C, N, and P biomass contents during stage III were 38.5, 6.5, and 1.4 %, respectively (Table 4). *Synechocystis aquatilis* was the predominant microalgae in this period, with *Microspora* sp. and *P. minima* present in similar proportions. The lipid content increased to 8.9±3.2 % during stage III, which was the maximum value recorded.

An increase in the liquid recirculation from 1.1 to 3.5 m^3 m^{-2} h^{-1} in stage IV (corresponding to a L/G increase in the AC from 0.9 to 2.9) resulted in an enhancement in RE_{CO2} up to 84±4 % without an increase in the O_2 concentration in the upgraded biogas (Fig. 4).

Despite the increase in RE_{CO2}, RE_C remained similar to the previous stage (19±3 %), which caused an IC concentration increase to 222±15 mg L^{-1}. RE_{TN} increased to 57±3 %, while RE_{Ps} and P_s concentrations were similar to stage III (45±10 % and 1.7±0.5 mg L^{-1}, respectively). The biomass productivity and TSS concentration in the HRAP during stage IV decreased slightly to 3.4±0.2 g m^{-2} day^{-1} and 0.22±0.01 g L^{-1}, respectively, with a $RE_{settler}$ of 95.5±1.5 % (Table 3). The C, N, and P biomass compositions were similar to previous stages (40.1, 7.1, and 1.3 %, respectively). *Synechocystis aquatilis* (67 %) was again the predominant microalga. *Microspora* sp. almost disappeared, while *P. minima* increased its predominance to 33 % of cells (Fig. 3). The lipid content decreased to 4.1±1.0 %.

Centrate dilution was increased to 1:70 during stage V to induce the nitrogen starvation conditions required for lipid accumulation. RE_{CO2}, RE_C, and IC concentrations of 82±2 %, 22±1 %, and 211±6 mg L^{-1}, respectively, were recorded. The lower nutrient load implied an increase in RE_{TN} to 70±6 % and a decrease in the effluent TN concentration to 5 mg N L^{-1}. Similarly, RE_{Ps} increased to 53±12 % with a concomitant decrease in P_s effluent concentration to 0.7±0.1 mg L^{-1}. Despite the lower nutrient load applied, biomass productivity and TSS concentration remained similar at 4.4±1.0 g m^{-2} day^{-1} and 0.29±0.03 g L^{-1}, respectively, with complete biomass removal in the settler (Table 3). Similar to previous operational stages, the C, N, and P biomass compositions were 42.5, 6.2, and 1.6 %, respectively. *P. minima* was identified as the dominant taxon (92 % of cells). A slightly higher lipid content of 7.4±0.4 % was achieved during stage V (Table 4).

The increase in the external liquid recirculation to 9.9 m^3 m^{-2} h^{-1} (L/G of 8.3 in the AC) in stage VI resulted in RE_{CO2} up to 99±0 % and resulted in an increase in the O_2 concentration of the upgraded biogas from 2.1±1.2 to 20.7±0.1 % (Fig. 4). Despite a slightly high increase in RE_C to 27±6 % being recorded, the IC concentration in the effluent increased to 228±15 mg L^{-1}. Complete TN removal was achieved in stage VI, which supported the occurrence of

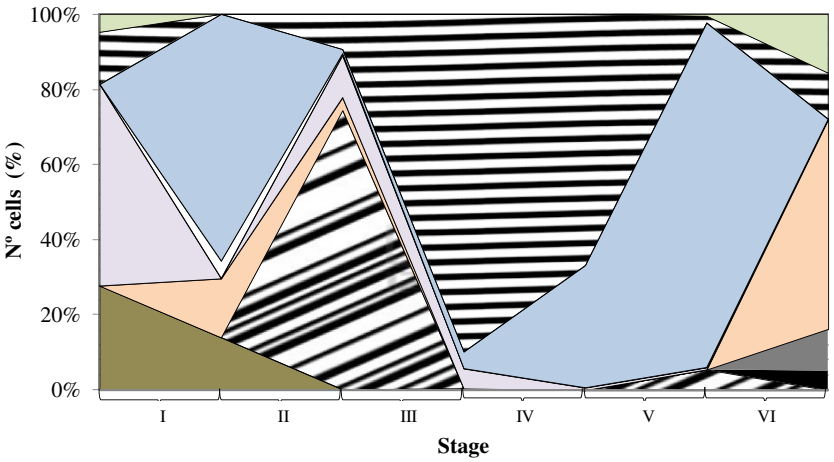

Fig. 3 The microalgae population structure in the HRAP during the six operational stages as a function of time. *Chroococcidiopsis* sp., *Cyanosarcina* sp., *Geitlerinema* sp., *Limnothrix mirabilis*, *Microspora* sp., *Mucidosphaerium pulchellum*, *Pseudanabaena minima*, *Scenedesmus* sp., *Synechocystis aquatilis*, and *Woronichinia* sp.

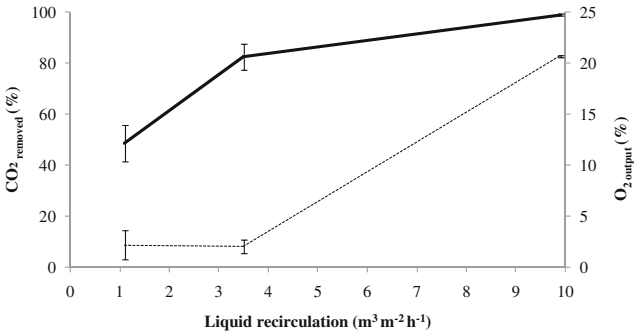

Fig. 4 Influence of the liquid recirculation flow rate on the CO_2-AC on CO_2 removal (*continuous line*) and O_2 concentration in the upgraded biogas (*discontinuous line*)

nitrogen deprivation conditions. Likewise, the high RE_{Ps} (82 ±3 %) recorded resulted in the lowest P-PO_4^{3-} concentrations in the HRAP (0.2 mg L^{-1}). Biomass productivity and TSS concentration remained at 3.6±0.1 g m^{-2} day^{-1} and 0.23± 0.02 g L^{-1}, respectively, with an $RE_{settler}$ of 97 % (Table 4). The C, N, and P compositions were 43.6, 7.5, and 1.4 %, respectively. Finally, the microalgae population during stage VI was characterized by a high biodiversity. Although *Limnothrix mirabilis* was the dominant species (57 %), *Geitlerinema* sp., *Synechocystis aquatilis*, and *Woronichinia* sp. were present at similar cell proportions (≈12–15 %). The lipid content of the algal–bacterial biomass decreased to 3.3± 0.7 %, which was the lowest recorded value.

Discussion

The evaporation rate in the HRAP was similar to outdoor systems (≈2–7 L m^{-2} day^{-1}), depending on the geographical area and the HRAP operational conditions (Guieysse et al. 2013; Murphy and Allen 2011) because of the high turbulence in the pond mediated by motor oversizing typical of lab- or pilot-scale systems (Mendoza et al. 2013). This high cultivation broth turbulence, together with the in situ O_2 produced by microalgae, maintained DO at 6–8 mg L^{-1}, well above the O_2 half-saturation constant for organic matter oxidation and nitrification (≥2 mg O_2 L^{-1}) (Metcalf et al. 2003). Likewise, this high turbulence avoided conditions where the algal cells remained in the dark for long periods of time.

The estimated dissolved CO_2 concentration in the liquid phase was calculated based on the pH and IC concentration in the cultivation broth of the HRAP and on the CO_2 aqueous equilibrium (Metcalf et al. 2003):

$CO_2(l)+H_2O(l)\leftrightarrow H_2CO_3\leftrightarrow HCO_3^-+H^+\leftrightarrow CO_3^{2-}+2H^+$ [pKa1 ($H_2CO_3\leftrightarrow HCO_3^-$)=6.35; pKa2 ($HCO_3^-\leftrightarrow CO_3^{2-}$)= 10.33]

According to these calculations, the estimated dissolved CO_2 concentration in the liquid phase decreased from 0.44 mg L^{-1} (stage I) to approximately 0.03 mg L^{-1} when the pH was increased to 9.5 in stage II. This tiny decrease in the dissolved CO_2 concentration in the bulk aqueous phase when pH was increased did not result in significant enhancements in the CO_2 concentration gradient from the CO_2 aqueous equilibrium in the column (the CO_2 gradient increased from 433.6±9.3 to 434.0±8.2 mg L^{-1}) and consequently in RE_{CO2} in the CO_2-AC (Fig. 5a, b). The estimated CO_2 equilibrium concentration in the liquid phase in the AC was calculated using the ideal gas equation and a Henry's nondimensional constant of 0.83 at 20 °C (Sander 1999), which resulted in a CO_2 equilibrium concentration of ≈434 mg CO_2 L^{-1} (Fig. 5a, b). Similarly, the decrease in the diffuser pore size (which resulted in higher gas–liquid interfacial areas) did not result in enhancements of RE_{CO2} because of the higher influence of the liquid recirculation flow rate on CO_2 removal, as discussed below. In this context, the superior biogas upgrading at higher external liquid recirculation rates was likely because of the higher net CO_2 absorption in the algal–bacterial recycling broth, as CO_2 mass transfer to the aqueous phase did not limit the upgrading process during stages I to III. On the other hand, the oxygen content in the upgraded biogas increased with increasing liquid recirculation rate above 3.5 m^3 m^{-2} h^{-1}, at concentrations well above regulatory limits in most European legislation for biomethane injection in natural gas grids (≤0.3 %) (BOE 2013). This high O_2 content in the biogas at stage VI would entail potential explosion hazards. In this context, the presence of high H_2S concentrations in the biogas and its rapid oxidation in the absorption column, together with the application of further operational strategies in this innovative biogas upgrading technology (e.g., feeding raw centrate or wastewater to the AC to deplete the O_2 present in the microalgal recycling stream via organic matter mineralization), are expected to lower these O_2 concentrations (Bahr et al. 2014; Serejo et al. 2015). The almost complete removal of CO_2 (99±0 %) at a L/G ratio of 8.3 was higher than the RE_{CO2} reported by Serejo et al. (2015) (≈80 %) at a L/G ratio of 10, which was determined as the optimum to achieve 100 % of H_2S removal and O_2 concentrations in the treated biogas of ≈1 %. In addition, it must be highlighted that the energy cost associated with the external recirculation was negligible compared to the energy requirements for mixing in the HRAPs (Bahr et al. 2014). Finally, it should be stressed that solubilization and biological oxidation of CH_4 would be expected when upgrading real biogas. However, preliminary tests carried out in our laboratory with simulated biogas showed average CH_4 losses of ≈1 % (on a mass basis) and a negligible effect of CH_4 on algal population activity (Serejo et al. 2015).

The main causes underlying the negative RE_{TOC} values recorded during the six operational stages were the low concentration and poor biodegradability of the dissolved organic carbon in the diluted centrates fed to the HRAP (Table 1) and the likely presence of TOC released by microalgal metabolism or TOC corresponding to biomass lysis (Posadas et al. 2013;

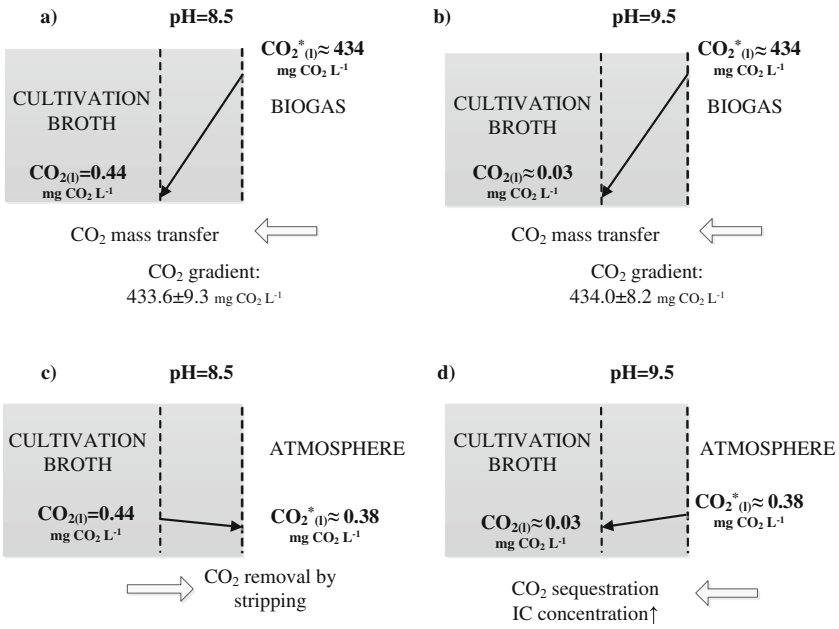

Fig. 5 Estimated CO_2 concentration gradients between the biogas and the external recirculating cultivation broth in the AC at a pH of 8.5 (**a**) and 9.5 (**b**) and between the cultivation broth in the HRAP and the atmosphere at a pH of 8.5 (**c**) and 9.5 (**d**)

Bahr et al. 2014; Dong et al. 2014; Serejo et al. 2015). At this point, it should be stressed that any photosynthetic biogas-upgrading system is a net bioconverter of dissolved IC into particulate organic carbon, and the active lysis of this structural organic carbon (often 10–15 % of the new microalgae biomass produced) generates significant amounts of dissolved recalcitrant organic carbon in the form of algal cell debris. On the other hand, the positive CO_2 concentration gradient established between the bulk liquid phase ($CO_{2(l)}$=0.44 mg CO_2 L^{-1}) and the atmosphere ($CO_2^{*}{}_{(l)}$=0.38 mg CO_2 L^{-1}) promoted CO_2 removal by stripping in stage I because only 17±6 % of the total carbon removed was recovered in the harvested biomass. The estimated aqueous CO_2 concentration in equilibrium with the atmosphere was also calculated using the ideal gas equation and a Henry's nondimensional constant of 0.83 at 20 °C (Sander 1999), which resulted in an equilibrium concentration of ≈0.38 mg CO_2 L^{-1} (Fig. 5c, d). However, the high pH value imposed from stage II onward compared to the reported optimum values of 8 for algal growth of 7–9 for wastewater treatment in HRAPs (Acién et al. 2012; Posadas et al. 2015) mediated CO_2 absorption from the atmosphere ($CO_{2(l)}$ ≈0.03 mg CO_2 L^{-1}) from stage II onward and prevented CO_2 removal by stripping, despite the higher IC concentrations present in the cultivation broth (Fig. 5c, d). Indeed, the total carbon recovered in the harvested biomass from stage II onward accounted for 100±5 % of the total C removed in the HRAP.

TN during stage I was removed by assimilation into biomass (15±8 %) and by N-NH_4^+ stripping (De Godos et al. 2009; Cai et al. 2013). Despite the pH being increased to 9.5 from stage II onward, 85±2 % of the TN removed was recovered in the harvested biomass. In this context, the complete N-NH_4^+ removal and the high nitrification activity supported by the high N-NO_3^- effluent concentrations at all operational stages (Table 3) indicated that N-NH_4^+ oxidation by nitrifying bacteria was faster than N-NH_4^+ volatilization. Similar results were obtained when treating raw domestic wastewater in an algal turf scrubber photobioreactor at HRTs of 5 to 10 days (Posadas et al. 2013). As a matter of fact, the RE_{TN} decrease during stages II and III was correlated to the increase in N-NO_3^- concentration (which prevented NH_4^+ volatilization). On the other hand, the higher IC availability could have mediated the slight increase in TN removal as a result of an enhanced biomass growth at stage IV, while the lower N loads supplied from stage V onward resulted in the higher RE_{TN} recorded. An increase in nitrification activity as a result of the higher IC availability was ruled out based on the steady decrease in nitrate concentration from stage IV onward.

RE_{Ps} also showed a significant increase compared to stages II, III, and IV when centrate dilution was increased. Phosphorus assimilation into biomass was the main removal mechanism during the six operational stages, with 98±3 % of the total removed P_s recovered in the harvested biomass (Cai et al. 2013). Phosphorus did not limit microalgae growth, which occurs at P_s concentrations below 0.2 mg L^{-1}.

The fact that the increase in RE_{CO2} did not result in higher biomass productivities was caused by the low nutrient loads fed into the system to increase biomass lipid content. Thus, these biomass productivities (maximum productivity of 5.4± 0.2 g m^{-2} day^{-1} in stage II), which could be increased by increasing the nutrient loads, were significantly lower than the typical productivities reported in outdoor HRAPs (≈15–20 g m^{-2} day^{-1}) (Chisti 2007). Nitrogen deprivation was the selected strategy to increase microalgae lipid content, which resulted in the low biomass productivities recorded. However, a balance between biomass lipid content and biomass

productivity should be targeted in real-scale applications (Feng et al. 2011). The enhanced biomass settleability (≈100 % $RE_{settler}$) achieved at the highest biomass concentrations (≥0.22 g TSS L^{-1}) was in agreement with the findings of Park et al. (2011b). In this context, microalgae-based wastewater treatment with effective biomass harvesting by settling represents a cost-effective alternative for algal biomass reutilization compared to conventional physical–chemical techniques, such as centrifugation or coagulation/flocculation (De Godos et al. 2011).

Similar C, N, and P biomass contents were recorded during the 225 days of experimentation regardless of the different C and nutrient loads applied, which confirms the constant composition of the algal–bacterial biomass cultivated in wastewater (Posadas et al. 2013). The C, N, and P biomass contents were in agreement with those reported by Dominguez Cabanelas et al. (2013) (C 43–56 %; N 2–9 %; P 1.4 %).

A high microalgal biodiversity was found regardless of the operational conditions tested. However, no significant correlation between cultivation conditions and the predominant microalgae species was clearly elucidated. The open design of the HRAP, together with the variations in characteristics of the fed diluted centrate, likely explained the high biodiversity and the rapid changes in the population structure (Devi et al. 2013). These results confirmed the difficulty of maintaining monoalgal species in open photobioreactors treating wastewaters (De Godos et al. 2009; García et al. 2000; Serejo et al. 2015).

The lipid content of the algal–bacterial biomass remained low despite the different carbon and nutrient supply strategies evaluated. The lipid contents obtained (2.9–11.2 %) were in the low reported range in axenic microalgae cultures (5–77 %, Chisti 2007) but were similar to those values reported in microalgae cultivated in wastewaters (2–23 %, Serejo et al. 2015; Sepúlveda et al. 2015). Contrary to previous findings (Toledo-Cervantes et al. 2013), nitrogen depletion in stages I and VI did not result in higher biomass lipid contents. The higher carbon availability during stages II and III compared to the start-up period likely resulted in an increase in biomass lipid content. Unfortunately, these contents were not sufficient to sustain a cost-effective wastewater to biodiesel process (≈30–40 %; Chisti 2007). This might be explained by the fact that only few of the species found in the HRAP are lipid producers in moderate concentrations. In this context, despite *Scenedesmus* sp. having been reported as the most efficient producer of lipids suitable for biodiesel among the identified microalgae, its abundance in the HRAP was low and limited to stage I (Mandal and Mallick 2012). The low light irradiances in this preliminary proof-of-concept study could have also contributed to the poor biomass lipid accumulation under nutrient limitation because some authors have reported enhanced lipid synthesis at increasing light irradiances (without reaching photosaturation conditions) under nitrogen-limited microalgae growth (Klok et al. 2013; Kandilian et al. 2014). In this context, the biomass harvested here could be used for other purposes than for biodiesel production. Among the potential uses of this residual algal biomass are biofertilization and as feedstock for the production of bioethanol and/or biogas (depending on its respective protein and carbohydrate contents) (Collet et al. 2011; Romero García et al. 2012; Wang et al. 2013; Hernández et al. 2015). Although the natural variations in solar irradiation, number of sun hours, and temperature are likely to hinder a fair comparison among the different experimental stages, a further evaluation of this technology must be carried out under outdoor conditions based on the promising results for biogas upgrading and wastewater treatment reported here.

In brief, complete CO_2 removal from biogas was achieved at liquid recirculation rates of 9.9 m^3 m^{-2} h^{-1}, which confirms the potential of this technology to photosynthetically upgrade biogas based on residual nutrients from anaerobic digesters. Assimilation into biomass represented the main N and P removal mechanisms at pH 9.5, regardless of the operational conditions. Biomass composition remained constant despite the rapid dynamics in microalgae population structure. The HRAP also supported an efficient biomass–effluent separation by settling (>95 %). Finally, the low lipid content (2.9–11.2 %) recorded throughout the different operational stages (even under nutrient limitation) showed the technical difficulty of developing cost-effective wastewater treatment for biodiesel processes, but the harvested biomass could be profitably applied for other uses, such as biofertilizers or bioethanol and biogas production.

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Feasibility study of biogas upgrading coupled with nutrient removal from anaerobic effluents using microalgae-based processes

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SUPPLEMENTARY MATERIAL

Table S1. Microalgal assemblage in the inoculum.

Microalga	No. of cells (%)	Volume (%)
Microspora sp.	53.5	21.6
Scenedesmus	27.8	67.4
Synechocystis aquatilis	13.9	6.0
Woronichinia sp.	4.7	5.0

Table S2. Microalgal assemblage in the HRAP during stage I.

Microalga	No. of cells (%)	Volume (%)
Limnothrix mirabilis	15.7	0.02
Mucidosphaerium pulchellum	4.7	9.5
Pseudanabaena minima	65.6	1.0
Scenedesmus sp.	14.1	89.5

Table S3. Microalgal assemblage in the HRAP during stage II.

Microalga	No. of cells (%)	Volume (%)
Chroococcidiopsis sp.	74.3	5.6
Limnothrix mirabilis	3.4	1.9
Microspora sp.	11.2	15.7
Mucidosphaerium pulchellum	0.5	10.1
Pseudanabaena minima	1.1	0.03
Scenedesmus sp.	0.2	64.3
Synechocystis aquatilis	9.4	2.5

Table S4. Microalgal assemblage in the HRAP during stage III.

Microalga	No. of cells (%)	Volume (%)
Microspora sp.	5.3	41.8
Pseudanabaena minima	4.4	0.8
Scenedesmus	0.4	48.9
Synechocystis aquatilis	89.8	8.5

Table S5. Microalgal assemblage in the HRAP during stage IV.

Microalga	No. of cells (%)	Volume (%)
Microspora sp.	0.7	66.5
Pseudanabaena minima	32.5	2.1
Synechocystis aquatilis	66.8	31.5

Table S6. Microalgal assemblage in the HRAP during stage V.

Microalga	No. of cells (%)	Volume (%)
Chroococcidiopsis sp.	5.5	49.1
Microspora sp.	0.6	44.8
Pseudanabaena minima	91.7	0.6
Synechocystis aquatilis	1.8	4.0
Woronichinia sp.	0.5	1.6

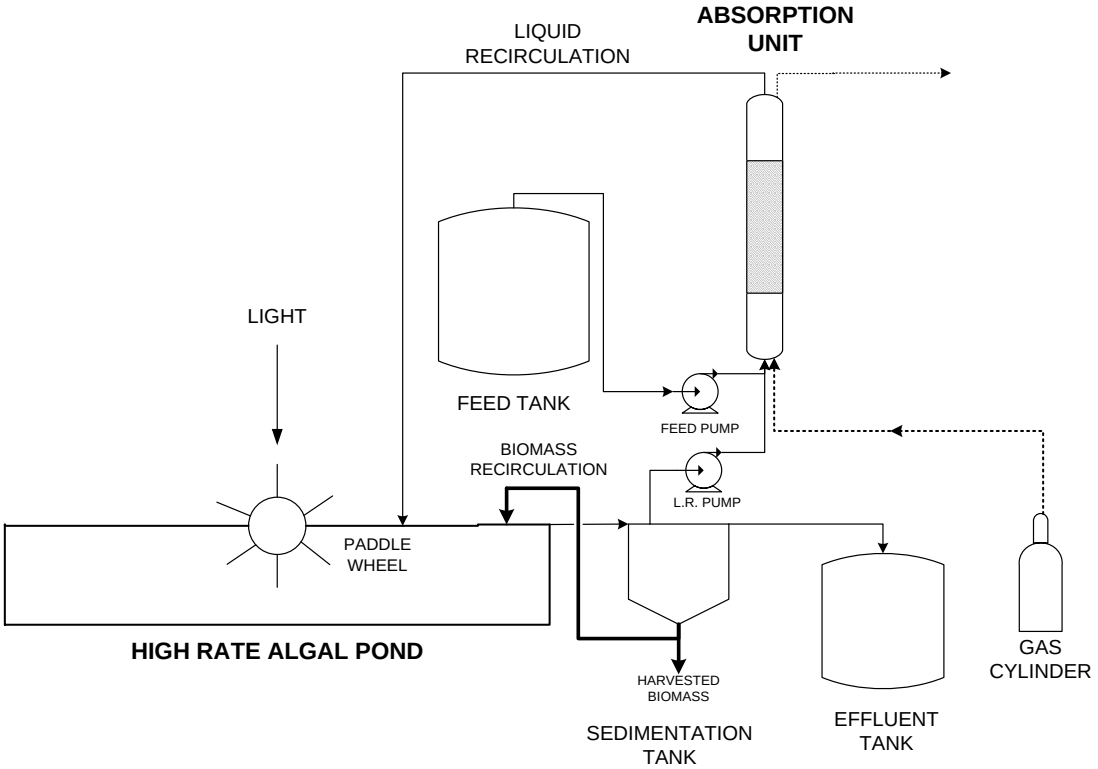
Table S7. Microalgal assemblage in the HRAP during stage VI.

Microalga	**No. of cells** (%)	**Volume** (%)
Chlorococcus sp.	0.4	60.0
Cyanosarcina sp.	2.8	13.0
Geitlerinema sp.	11.4	3.4
Limnothrix mirabilis	56.9	0.7
Synechocystis aquatilis	12.7	12.3
Woronichinia sp.	15.8	10.7

Minimization of Biomethane Oxygen Concentration during Biogas Upgrading in Algal-Bacterial Photobioreactors

Posadas E., Serejo M. L., Blanco S., Pérez R., García Encina P.A., Muñoz R. (2015), Algal Res. 12: 221-229.

Chapter 7.2



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Minimization of biomethane oxygen concentration during biogas upgrading in algal–bacterial photobioreactors

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ABSTRACT

Novel operational strategies to reduce the O_2 concentration in the upgraded biogas were evaluated in a 180 L algal–bacterial photobioreactor interconnected to a 2.5 L external absorption column during the simultaneous treatment of diluted anaerobically digested or raw vinasse and biogas upgrading. The lowest biomethane O_2 levels (0.7 ± 0.2%) were recorded when raw vinasse was fed directly into the absorption column, which resulted in CO_2 and H_2S removals from biogas of 72 ± 1% and 100 ± 0%, respectively. Process operation at a Hydraulic Retention Time (HRT) of 7 d under the above configuration also supported the maximum total carbon, nitrogen and phosphorus removals of 72 ± 4%, 74 ± 3% and 78 ± 5%, respectively. Biomass productivity ranged from 11.4 ± 1.8 to 13.5 ± 2.2 g m^{-2} d^{-1} during microalgae cultivation in diluted anaerobically digested vinasse, while this productivity increased to 16.9 ± 0.7 g m^{-2} d^{-1} when feeding diluted raw vinasse. The good settling characteristics of the algal–bacterial flocs resulted in an average harvesting efficiency of 98.6 ± 0.5% at a HRT in the settler of 23 min, regardless of the treated vinasse. The morphological and molecular characterization of the microbial communities showed a high microalgae diversity and bacterial species richness, regardless of the operational conditions (Shannon–Wiener indices ranging from 2.8 to 3.3).

1. Introduction

The total electricity produced in the European Union in 2013 through primary biogas production accounted for 52.3 TWh [1]. Biogas from the anaerobic digestion of renewable feedstocks (such as agroindustrial or municipal organic solid wastes) constitutes a potential biofuel source able to reduce the current fossil fuel dependence of our society [2]. Biogas is a gas rich in CH_4 (40-75%) and CO_2 (25–60%), with other components such as H_2S (0.005–2%), N_2 (0–2%), O_2 (0–1%) and NH_3 (<1%) present at significantly lower concentrations [3]. CO_2 removal from biogas would entail a decrease in its transportation and compression costs, while the removal of H_2S would reduce its toxic, corrosive and malodorous nature [4]. In this context, a removal of CO_2 and H_2S to achieve CH_4 concentrations over 80–96% and H_2S levels below 5 mg m^{-3} is required for biomethane injection into natural gas grids and use as a vehicle fuel [5]. Conventional physical/chemical or biological technologies often tackle CO_2 or H_2S removal into two sequential steps [3,6]. Otherwise, processes such as water/chemical scrubbing and membrane separation, which allow for a simultaneous CO_2 and H_2S removal from biogas, exhibit high environmental impacts and operating costs, respectively [3,7]. In this context, algal–bacterial symbiosis allow for a simultaneous CO_2 and H_2S removals in an innovative, environmentally friendly and low-cost process compared to conventional methods [8].

Microalgae-based processes for biogas upgrading are characterized by the simultaneous photosynthetic CO_2 consumption by microalgae in the presence of light and the oxidation of H_2S to sulfate by sulfur oxidizing bacteria using the O_2 produced from microalgal photosynthesis [8]. The economic and environmental sustainability of this biotechnology can be enhanced with the use of wastewaters as a free water and nutrient source for microalgae and bacteria growth [9]. Despite the promising results obtained so far in terms of biogas upgrading and wastewater treatment performance, the desorption of the photosynthetically produced O_2 from the algal cultivation broth to the upgraded biogas severely challenges the application of this novel biotechnology [10]. Thus, while the upper O_2 concentration limit for injection of the upgraded biogas into natural gas networks stands at 0.2–1% in most international legislations, O_2 levels ranging from 2–24% have been typically reported in biogas-upgrading photobioreactors [8,11]. In this context, Posadas et al. [10] recorded CO_2 removals from synthetic biogas of 99% and O_2 concentrations in the upgraded biogas of ≈20% in a 180 L open photobioreactor treating diluted centrates, while Serejo et al. [12] recorded CO_2 and H_2S removals of ≈80% and 100%, respectively, and O_2 concentrations in the upgraded stream ranging from 2 ± 1% to 1 ± 0% in a similar

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Nomenclature

AC	absorption column
ADV	anaerobically digested vinasse
B	biomass (g)
C	carbon
COD	chemical oxygen demand (mg L^{-1})
DO	dissolved oxygen (mg L^{-1})
HRT	hydraulic retention time (d)
HRAP	high rate algal pond
IC	inorganic carbon (mg L^{-1})
$L_{liquid\ recirculation}/G_{biogas}$	ratio liquid (liquid recirculation) and gas (biogas) phases
N	nitrogen
P	phosphorus
P_s	soluble phosphorus (mg L^{-1})
RE	removal efficiency (%)
RV	raw vinasse
SVI	sludge volumetric index (mL g^{-1})
TC	total carbon (mg L^{-1})
TIC	total inorganic carbon (mg L^{-1})
TN	total nitrogen (mg L^{-1})
TOC	total organic carbon (mg L^{-1})
TSS	total suspended solids (mg L^{-1})
W	areal biomass productivity (g $m^{-2}_{surface\ HRAP}$ d^{-1})

photobioreactor by treating diluted anaerobically digested vinasse (ADV). Likewise, Converti et al. [13] reported O_2 concentrations ranging from 10 to 24% during the upgrading of real biogas in a bubble column photobioreactor. These high O_2 levels entail a potential explosion hazard and prevent the injection of the upgraded biogas into natural gas networks. Membranes or low temperature pressure swing adsorption (PSA) are typically used for O_2 removal from biogas, but these technologies present very high operating costs [3]. Unfortunately, the number of studies focused on the reduction of O_2 concentrations in biomethane during the simultaneous removal of CO_2 and H_2S in algal–bacterial processes is scarce.

The present study assessed the effectiveness of different operational strategies to reduce the O_2 concentration in the upgraded biogas in a 180 L high rate algal pond (HRAP) treating ADV and interconnected to an external CO_2/H_2S absorption column (AC). The removal of CO_2 and H_2S from a synthetic biogas and the potential of this novel biotechnology for carbon and nutrient removal from ADV were also evaluated. Finally, the dynamics of the structure of microalgae and bacteria populations in the HRAP were investigated. This study constitutes, to the best of our knowledge, the first evaluation by molecular techniques of the bacterial assemblage dynamics in this innovative photosynthetic biogas upgrading process.

2. Materials and methods

2.1. Biogas and vinasse wastewater

A synthetic biogas mixture, composed of CO_2 (29.5%), H_2S (0.5%) and CH_4 (70%), was purchased from Abello Linde (Spain). ADV and raw vinasse (RV) wastewaters were periodically collected from the anaerobic wastewater treatment line of a food industry located in Valladolid (Spain) and stored at 4 °C prior to use. The final composition of the feed wastewaters was subjected to the variations of the received wastewaters depending on the seasonal period. ADV and RV were diluted twelve times with tap water prior feeding to the HRAP in order to avoid microalgae inhibition due to their high N-NH_4^+ concentrations [14] (Table 1). ADV dilution was set according to Serejo et al. [12], while RV dilution was set to maintain the same nutrient loading rate into the HRAP.

2.2. Experimental set-up

The experimental set-up consisted of a 180 L HRAP with an illuminated surface of 1.2 m^2 (202 cm length × 63 cm width × 15 cm depth) and two water channels divided by a central wall, interconnected to a 2.5 L (Ø = 4.4 cm; height = 165 cm) external absorption column. The design of the column was chosen according to the results reported by Bahr et al. [8] during the optimization of the performance of an AC in a similar experimental set-up. A countercurrent operation would have entailed the exposure of the recycling cultivation broth with the highest DO to the biomethane exiting the AC, which would have avoided the biological O_2 consumption in the column. The HRAP and AC were interconnected via an external liquid recirculation of the microalgae-bacteria broth from an 8 L settler, which was located at the outlet of the HRAP (Fig. 1). The internal recirculation velocity of the HRAP cultivation broth was ≈ 20 cm s^{-1}, which was provided by the continuous rotation of a 6-blade paddlewheel [8]. HRAP illumination was performed using 33 fluorescent bulbs (20 W, DUOLEC E27, Portugal) and 12 Gro-lux fluorescent lamps (Sylvania, Germany). The absorption unit consisted of a bubble column provided with a metallic sparger (2 μm pore size) located at its bottom. The HRAP was initially filled with 910 mg total suspended solids (TSS) L^{-1} of a consortium of microalgae/cyanobacteria (henceforth referred to as microalgae) and bacteria treating 12× diluted anaerobically digested vinasse wastewater in a similar HRAP. The microalgae inoculum composition was (% of cells): *Planktolynga brevicellularis* (81%), *Stigeoclonium tenue* (14%) and *Limnothrix planktonica* (5%). The system was operated indoors at the Department of Chemical Engineering and Environmental Technology of University of Valladolid (Spain) at 23 ± 1 °C.

2.3. Operational conditions

The hydraulic retention time (HRT) in the HRAP was maintained constant at a typical value for wastewater treatment in HRAPs of 7.4 ± 0.2 d during the entire experimental period [15,16]. The external microalgae broth recirculation was maintained at 475 ± 8 L d^{-1} and the continuos synthetic biogas flow rate in the AC at 44.4 ± 1.7 L d^{-1}, which resulted in a $L_{liquid\ recirculation}/G_{biogas}$ ratio of 10.7 ± 0.4. The gas flow rate and the $L_{liquid\ recirculation}/G_{biogas}$ ratio were set according to Serejo et al. [12]. The system was operated under light:dark cycles of 16:8 h at 104 ± 25 μmol m^{-2} s^{-1} during the illuminated period (7:00–23:00).

Four operational strategies, corresponding to stages 1, 2A, 2B and 3, were tested in order to reduce the oxygen concentration in the upgraded biogas. During stage 1, diluted ADV was fed directly into the cultivation broth of the HRAP (Fig. 1a). During stage 2A, the diluted ADV was mixed with the external liquid recirculation stream and fed into the AC to promote O_2 consumption by bacteria in the absorption unit, which would ultimately minimize the O_2 content in the upgraded biogas (Fig. 1b). Similarly, the ADV was mixed with the external liquid recirculation without dilution with tap water to boost the kinetics of bacterial organic matter oxidation (and therefore O_2 consumption) in the AC in stage 2B (Fig. 1c), while the tap water previously used for ADV dilution was directly fed into the HRAP. In stage 3, the experimental set-up was operated using the same configuration evaluated in stage 2B with RV instead of ADV due to its higher organic matter content and biodegradability. Each operational stage was maintained for approximately 28 d (≈4 × HRT), except stage 2 which was divided into 2A and 2B and maintained for ≈14 d (≈2 × HRT) based on their similar operational conditions. The areal carbon, nitrogen and phosphorus loads supplied to the HRAP in the ADV and synthetic biogas mixture during stages 1, 2A and 2B were 5 ± 1 g C m^{-2} d^{-1}, 1.4 ± 0.1 g N m^{-2} d^{-1} and 0.025 ± 0.002 g P m^{-2} d^{-1}, respectively, while during stage 3 these values (raw vinasse + synthetic biogas mixture)

corresponded to 12 ± 1 g C m^{-2} d^{-1}, 1.4 ± 0.1 g N m^{-2} d^{-1} and 0.029 ± 0.001 g P m^{-2} d^{-1}, respectively.

2.4. Sampling and analytical procedures

2.4.1. Environmental parameters

Ambient and cultivation broth temperatures, input and output flowrates, dissolved O_2 concentration (DO) and pH in the cultivation broth were daily monitored, while the light intensity at the HRAP surface was recorded under steady state operation. Temperature and dissolved oxygen concentration in the cultivation broth were determined using an OXI 330i oximeter (WTW, Germany). An Eutech Cyberscan pH 510 (Eutech instruments, The Netherlands) was used for pH determination in the HRAP. The photosynthetic active radiation (PAR) was measured with a LI-250A light meter (LI-COR Biosciences, Germany).

2.4.2. Gas phase

Gas samples of 100 μL of synthetic biogas (inlet of the AC) and upgraded biogas (outlet of the AC) were withdrawn twice a week in order to monitor the concentrations of CO_2, H_2S, CH_4, O_2 and N_2 (Fig. 1). The inlet and outlet biogas flow rates in the AC were also measured to accurately determine both CO_2 and H_2S removals. The gas CO_2, H_2S, CH_4, O_2 and N_2 concentrations were determined using a Varian CP-3800 GC-TCD (Palo Alto, USA) equipped with a CP-Molsieve 5A (15 m × 0.53 mm × 15 μm) and a CP-Pora BOND Q (25 m × 0.53 mm × 15 μm) columns.

2.4.3. Liquid phase

Liquid samples of 200 mL from the influent wastewater and treated effluent after settling were withdrawn twice a week (Fig. 1) to monitor the concentrations of total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), N-NH_4^+, N-NO_2^-, N-NO_3^-, phosphorus (P_s) and pH. Chemical Oxygen Demand (COD) concentration was only measured at steady state, which was considered to be reached under constant concentrations of the monitored parameters. COD, TOC, IC, TN, N-NH_4^+, N-NO_2^-, N-NO_3^- and P_s concentrations corresponded to the soluble phase, and required liquid sample filtration through 0.20 μm nylon filters prior to analysis. The concentrations of dissolved TOC, IC and TN were measured using a Shimadzu TOC-VCSH analyzer (Japan) coupled with a TNM-1 chemiluminescence module. N-NH_4^+ concentration was determined with an ammonium specific electrode Orion Dual Star (Thermo Scientific, The Netherlands). The concentrations of N-NO_3^- and N-NO_2^- were quantified by HPLC-IC according to Posadas et al. [17]. The concentration of soluble phosphorus was determined spectrophotometrically using the ammonium molybdate method (Spectrophotometer U-2000, Hitachi, Japan). All analyses, including COD, were carried out according to Standard Methods [18]. Biogas and liquid sampling was always conducted at 9:00 a.m. along the entire experimental period, which was considered representative of the process based on the constant microalgae activity after two hours of illumination and the high HRT that softened the variations in IC concentrations in the cultivation broth potentially caused by IC accumulation in the dark period and its consumption by microalgae during the illuminated period.

2.4.4. Biomass characterization

Likewise, liquid samples of 100 mL were drawn from the cultivation broth twice a week to monitor the algal–bacterial TSS concentration. The sludge volume index (SVI) of the algal–bacterial broth was also determined in duplicate under steady state operation. TSS and SVI analysis were carried out according to Standard Methods [18].

The high external liquid recirculation flow rate resulted in a high biomass accumulation in the settler, which entailed the need of a daily settled biomass recirculation to the HRAP to avoid biomass wash-out (Fig. 1).

2.4.4.1. Elemental composition. The algal–bacterial biomass harvested in the settler was dried for 24 h at 105 °C in order to determine its elemental (C, N and P) composition at steady state. The determination of the C and N content of the algal–bacterial biomass was conducted in a LECO CHNS-932 analyzer, while phosphorus content was determined spectrophotometrically after acid digestion in a microwave based on the internal procedure of the Instrumental Technical Laboratory of Valladolid University according to Standard Methods [18].

2.4.4.2. Microalgae population determination. The identification, quantification and biometry measurements of the microalgae assemblage at steady state were performed by microscopic examination (OLYMPUS IX70, USA) of biomass samples (fixed with lugol acid at 5% and stored at 4 °C prior to analysis) according to Sournia [19].

2.4.4.3. Bacterial community determination and Shannon–Wiener diversity indices' calculation. Biomass samples were also withdrawn under steady state operation in each stage and stored immediately at −20 °C in order to evaluate the richness and composition of the bacterial community. The V6-V8 regions of the bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the universal bacterial primers 968-F-GC and 1401-R (Sigma- Aldrich, St. Louis, MO, USA) [20]. The PCR mixture (50 μL) contained 2 μL of each primer (10 ng μL^{-1} each primer), 25 μL of BIOMIX ready-to-use 2× reaction mix (Bioline, Ecogen), PCR reaction buffer and deoxynucleotide triphosphates (dNTPs), 2 μL of the extracted DNA and Milli-Q water up to a final volume of 50 μL. PCR was performed in a iCycler Thermal Cycler (Bio Rad Laboratories, Inc) with the following thermo-cycling program for bacterial amplification- 2 min of pre-denaturation at 95 °C, 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 45 s, and elongation at 72 °C for 1 min, with a final 5-min elongation at 72 °C. Size and yield of PCR products were estimated using a 2000-bp DNA ladder, Hypperladder II (Bioline, USA Inc) in 1.8% agarose gel (w/v) electrophoresis and GelRed Nucleic Acid Gel staining (Biotium).

The Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the amplicons was performed on 8% (w/v) polyacrylamide gels with a urea/formamide denaturing gradient of 45–65% [21]. Electrophoresis was performed with a D-Code Universal Mutation Detection System (Bio Rad Laboratories, Inc) in 0.5 × TAE buffer at 60 °C and 85 V for 16 h. The gels were stained with GelRed Nucleic Acid Gel (1:10,000 dilution; Biotium) for 1 h 30 min.

The sequencing and DNA sequence analysis were carried through the excision of individual bands from the DGGE gel with a sterile blade, resuspended in 50 μL of ultra pure water, and maintained at 60 °C for 1 h to allow DNA extraction from the gel. A volume of 5 μL of the supernatant was used for reamplification with the original primer

Table 1
Composition of the diluted anaerobically digested vinasse and diluted raw vinasse wastewater.

Diluted wastewater	COD ($mg L^{-1}$)	TOC ($mg L^{-1}$)	IC ($mg L^{-1}$)	TN ($mg L^{-1}$)	N-NH_4^+ ($mg L^{-1}$)	P_s ($mg L^{-1}$)	TSS ($g L^{-1}$)	pH
Anaerobically digested vinasse	243 ± 22	107 ± 7	157 ± 17	68 ± 1	58 ± 7	1.4 ± 0.1	0.2 ± 0.1	7.9 ± 0.2
Raw vinasse	921 ± 175	536 ± 86	67 ± 8	67 ± 6	59 ± 6	1.6 ± 0.1	0.5 ± 0.1	7.5 ± 0.1

Neither NO_2^- nor NO_3^- were detected in the wastewaters.

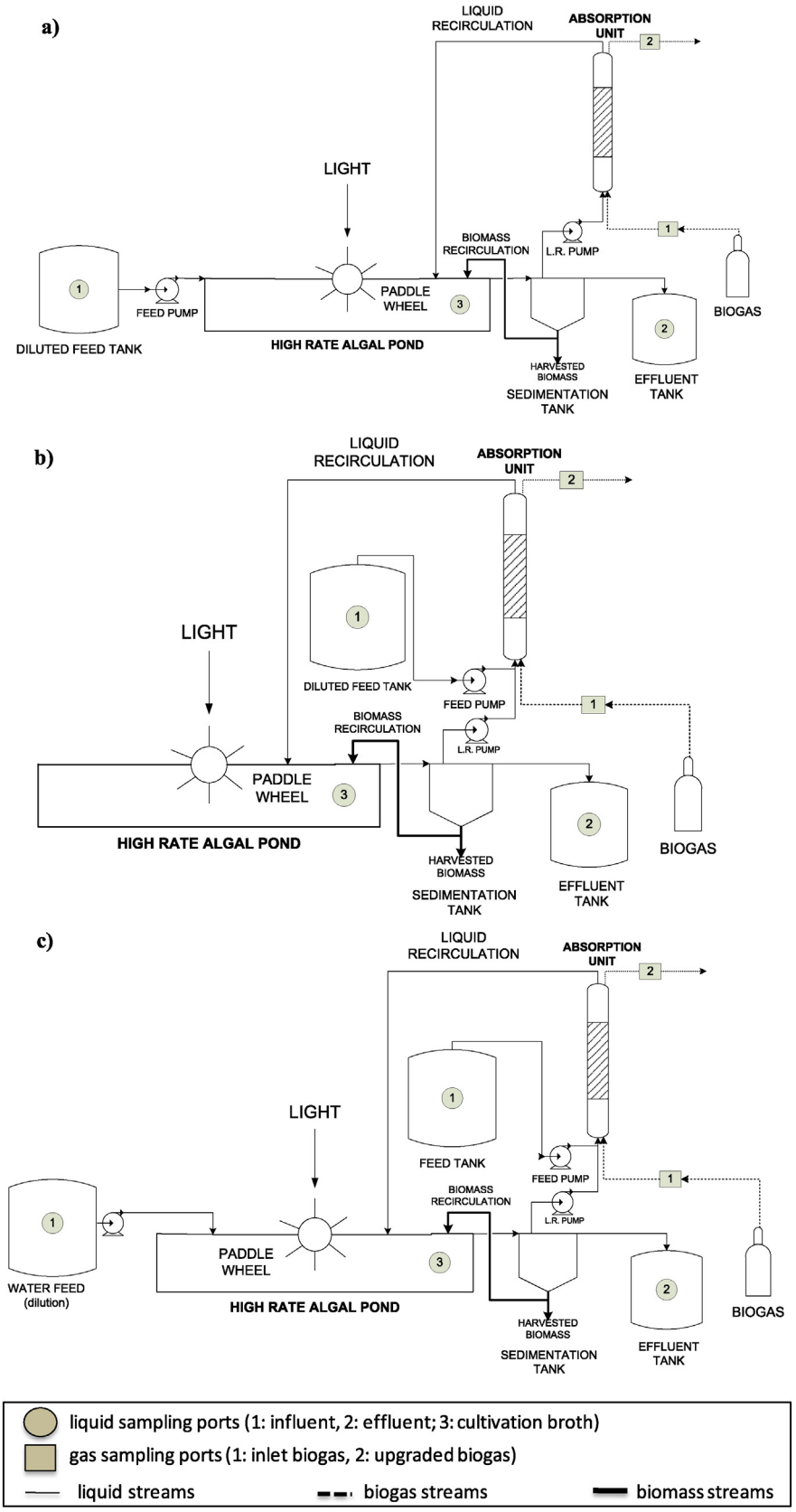

Fig 1. Schematic diagram of the continuous photosynthetic biogas upgrading and nutrient removal experimental set-up. a) Initial configuration (stage 1); b) Modified configuration I (stage 2A); c) Modified configuration II (stages 2B, 3). L.R. stands for liquid recirculation.

sets. Before sequencing, PCR products were purified with the GenElute PCR DNA Purification Kit (Sigma-Aldrich, St. Louis, MO, USA). The taxonomic position of the sequenced DGGE bands was obtained according to Frutos et al. [22]. Sequences were deposited in GenBank Data Library under accession numbers KR185739–KR185757. Bacterial DGGE profiles were compared using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). The gels were normalized by using internal standards. After image normalization, bands were defined for each sample using the bands search algorithm within the program. The software carries out a density profile analysis for each lane, detects the bands, and calculates the relative contribution of each band to the total band intensity in the lane. Similarity indices within the bacterial populations were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient [23], and were subsequently used to depict a dendrogram by using UPGMA clustering with error resampling (500 resampling experiments). Peak heights in the densitometric curves were also used to determine the Shannon–Wiener diversity indices according to the next equation:

$$H = -\sum[P_i \ln(P_i)] \quad (1)$$

where P_i is the importance probability of the bands in a lane ($P_i = n_i/n$, n_i is the height of an individual peak and n is the sum of all peak heights in the densitometric curves).

2.5. Calculations

Process performance was characterized by the steady state removal efficiency (RE) of CO_2 and H_2S from biogas; the overall RE of total carbon (TC) and total inorganic carbon (TIC) from biogas and wastewater; the RE of COD, TOC, TN, N-NH_4^+ and P_s from wastewater; the TSS removal efficiency in the settler ($RE_{settler}$); the areal biomass productivity (W); the biomass harvested and collected ($B_{harvested}$ and $B_{collected}$) every day in the settler and the overall mass balances of C, N and P. The calculation procedures for the above referred parameters are detailed in the supplementary materials section. The final results obtained throughout the four operational stages were provided as the average values recorded for 6 consecutive days during each steady state operation with their corresponding standard deviation.

2.6. Statistical treatment

An analysis of variance (ANOVA) with a Fisher's least significant difference test using a 95% confidence interval was used to assess any significant influence of the operational configuration on both the performance of biogas upgrading and wastewater treatment, and the productivity and characteristics of the biomass harvested under steady state at each operational stage.

3. Results and discussion

3.1. Environmental conditions in the HRAP

The temperature of the algal–bacterial broth remained constant at 24 ± 1 °C regardless of the operational stage, which was optimum for the cultivation of microalgae and bacteria in wastewaters [16]. Larger variations occurred in the average water evaporation losses recorded along the different operational stages, which ranged from 4.4 ± 1.4 to 7.3 ± 0.2 L m^{-2} d^{-1} (Table 2). These values were comparable to those estimated by Guieysse et al. [24] in outdoors HRAPs operated at 7 d of HRT and located in Arid, Mediterranean, Subtropical, Temperate and Tropical regions, which corresponded to water evaporations of 6.2, 3.6, 3.2, 2.0 and 1.3 L m^{-2} d^{-1}, respectively. The main cause of the high water evaporation losses in our particular HRAP was the high turbulence as a consequence of its pilot scale design, where the paddlewheel engine was oversized [25]. The highest DO concentration recorded (7 ± 1 mg O_2 L^{-1}) was not inhibitory for microalgae activity [26] and the lowest DO concentration (3 ± 1 mg O_2 L^{-1}) was high enough to support a successful organic matter oxidation and NH_4^+ nitrification (>2 mg O_2 L^{-1}) [27]. Likewise, based on the steady H_2S load and the $L_{liquid\ recirculation}/G_{biogas}$ applied, a minimum DO of 0.9 mg O_2 L^{-1} was required for a complete H_2S oxidation in the absorption column. Thus, despite the lower recorded DO concentrations compared to axenic microalgae cultures [28] or HRAPs devoted to wastewater treatment under outdoors conditions [15], the DO concentrations allowed successful microalgae-bacteria symbiotic interactions. Despite the differences in pHs and IC concentrations between the diluted ADV and RV fed into the HRAP (Table 1), the pH in the cultivation medium remained constant at ≈8, which has been reported as an optimum value for microalgae growth [28] (Table 2). The rather constant microalgal–bacterial activity and the high buffer capacity of the cultivation medium as a result of the high IC concentrations (which ranged from 71 ± 5 to 124 ± 2 mg L^{-1}) likely supported the constant pH levels without an automatic control (Table 2).

Table 2
Steady state water evaporation losses in the HRAP, dissolved oxygen concentration and pH of the cultivation broth, effluent dissolved carbon (TOC and IC) and chemical oxygen demand (COD), nitrogen (TN, N-NH_4^+ and N-NO_3^-) and phosphorus (P_s) concentrations during the four tested operational stages.

Parameters	Stage			
	1	2A	2B	3
Evaporation losses (L m^{-2} d^{-1})	6.4 ± 1.5	4.4 ± 1.4	5.1 ± 0.5	7.3 ± 0.2
DO (mg L^{-1})	7.1 ± 0.9	5.3 ± 0.5	4.6 ± 0.4	2.9 ± 0.6
pH_{HRAP}	8.0 ± 0.1	7.9 ± 0.2	8.1 ± 0.1	8.0 ± 0.1
Effluent TOC (mg L^{-1})	85 ± 1	91 ± 9	90 ± 8	105 ± 9
Effluent IC (mg L^{-1})	71 ± 5	82 ± 10	82 ± 8	124 ± 2
Effluent COD (mg L^{-1})	232 ± 40	166 ± 2	172 ± 33	143 ± 6
Effluent TN (mg L^{-1})	86 ± 6	74 ± 1	73 ± 5	24 ± 2
Effluent N-NH_4^+ (mg L^{-1})	0 ± 0	0 ± 0	0 ± 0	1.4 ± 1.0
Effluent N-NO_3^- (mg L^{-1})	70 ± 10	63 ± 10	57 ± 9	13 ± 2
Effluent P_s (mg L^{-1})	1.0 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	0.5 ± 0.1

3.2. CO_2 and H_2S removal and oxygen concentration in the biomethane

CO_2-REs of 79 ± 4%, 77 ± 5%, 78 ± 5% and 72 ± 1%, which corresponded to CO_2 concentrations of 6.8 ± 0.9%, 8.0 ± 1.6%, 6.6 ± 0.7% and 9.2 ± 0.5% in the upgraded biogas, were recorded during stages 1, 2A, 2B and 3, respectively (Fig 2a). The significantly lower CO_2-RE during stage 3 compared to the initial stage was likely due to the higher biodegradable organic matter load supplied to the AC in stage 3, which was oxidized to CO_2 thus reducing CO_2 absorption (Fig. 1).

A complete H_2S removal was achieved regardless of the operational stage. The high solubility of H_2S in the cultivation medium due to its high adimensional Henry's constant (2.44 compared to 0.83 for CO_2), together with the above mentioned high DO concentrations supported this successful H_2S removal [8,29].

The O_2 concentrations recorded in the upgraded biogas corresponded to 1.2 ± 0.2%, 1.2 ± 0.2%, 1.1 ± 0.4% and 0.7 ± 0.2% during stages 1, 2A, 2B and 3, respectively (Fig. 2b). The higher O_2 requirements for complete organic matter oxidation during RV feeding into the AC (stage 3) resulted in biomethane O_2 concentrations in compliance with the requirements of most international legislations for injection of the upgraded biogas in natural gas networks (0.2–1%) [5]. This biomethane O_2 concentration was significantly lower than that recorded by Posadas et al. [10], which ranged from 2.0 ± 1.0% to 20.7 ± 0.1%, and by Serejo et al. [12], which averaged 2 ± 1%. Likewise, the O_2 concentrations recorded here were significantly lower than those achieved by Mann et al. [30] in a 1 L enclosed tubular photobioreactor supporting CO_2 and H_2S-REs of 97% and 100%, respectively, with biomethane O_2 concentrations ranging from 18-23%. Similarly, the biomethane O_2

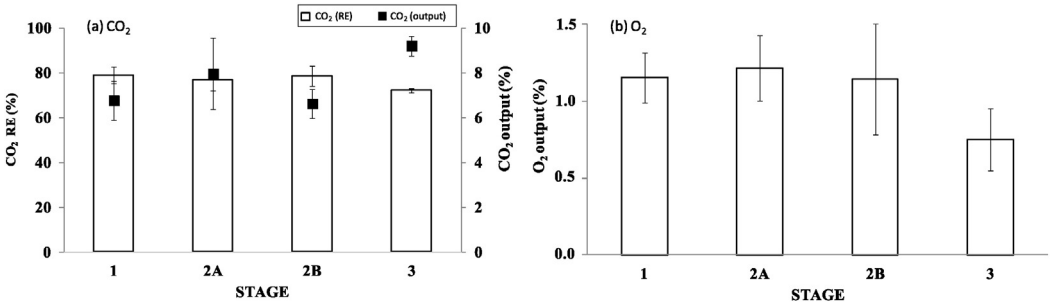

Fig 2. Influence of the operational strategy evaluated on a) CO_2 removal efficiency (main axis) and CO_2 concentration in the upgraded biogas (secondary axis); and b) oxygen concentration in the upgraded biogas. Vertical bars represent standard deviation.

levels reported here were also lower than the 10 to 24% recorded in a 1 L photobioreactor during biogas upgrading by *Arthrospira platensis* [13].

Despite these promising results, N_2 concentrations in the upgraded biogas during stages 1, 2A, 2B and 3 were 7.2 ± 2.0%, 6.1 ± 0.6%, 5.9 ± 1.1% and 6.0 ± 0.9%, respectively. These high concentrations, as a result of the high $L_{liquid\ recirculation}/G_{biogas}$ ratios, decreased the final CH_4 concentration in the upgraded stream down to 81 ± 2% (with a CH_4 solubilization in the aqueous phase ~1%), which was below the minimum threshold required for biomethane injection into natural gas grids [5]. Similar technical issues are encountered in high pressure (8–10 bar) water scrubbing units for biogas upgrading in which, despite their successful and simultaneous CO_2 and H_2S removals of ≥80% and 100%, respectively, the high amounts of N_2 (and O_2) desorbed into the treated biogas must be addressed [31]. Therefore, further design and operational strategies must be investigated in order to increase CO_2 removal while minimizing N_2 desorption [31]. Process optimization based on control strategies coupling biogas supply to microalgae activity (and therefore to daily variations in solar irradiance) must be carried out prior to full scale implementation in order to maximize the C-CO_2 recovered in the harvested biomass [15].

3.3. Wastewater treatment

TOC-REs of 41 ± 7%, 42 ± 6% and 44 ± 3% and COD-REs of 36 ± 3%, 45 ± 5% and 50 ± 5% were recorded in stages 1, 2A and 2B, respectively (Fig. 3a). Under these operational conditions, TOC and COD effluent concentrations ranged from 85 ± 1 to 91 ± 9 mg TOC L^{-1} and from 166 ± 2 to 232 ± 40 mg O_2 L^{-1}, respectively (Table 2). The slight but significant increase in COD-REs from stages 1 to 2B was likely due to the higher biodegradability of the received ADV wastewater rather than to the modification in the feeding mode (Fig. 1). This higher biodegradability resulted into a slightly higher O_2 consumption during stages 2A and 2B, which explained the DO concentration decrease from 7 to 5 mg O_2 L^{-1} (Table 2). The TOC and COD-REs during stage 3 were 85 ± 2 and 88 ± 2%, respectively, and resulted in TOC and COD effluent concentrations of 105 ± 9 mg TOC L^{-1} and 143 ± 6 mg O_2 L^{-1}, respectively. The highest concentration and biodegradability of the organic matter in RV compared to ADV wastewater entailed higher O_2 consumptions for organic matter stabilization and, therefore, lower DO concentrations in the cultivation medium (Table 2). These results confirmed the cost-effectiveness of algal–bacterial processes for organic matter stabilization in wastewaters [32].

TIC and TC-REs (considering the C-CO_2 removals above discussed) remained constant during stages 1, 2A and 2B at average values of 61 ± 5% and 51 ± 5%, respectively (Fig. 3a). However, negative TIC-REs were recorded during stage 3 based on the lower IC influent concentrations and the accumulation of IC in the cultivation medium as a result of the higher TOC oxidation (Tables 1, 2), while TC-REs increased to 72 ± 4% mainly driven by the higher TOC-RE (Fig. 3a). Under these operational conditions, the carbon mass balance calculation revealed that 65 ± 9% of the TC removed from the wastewater and biogas was recovered in the harvested biomass during stages 1, 2A and 2B, while this value decreased to 29 ± 7% in stage 3. This result could be explained taking into account the higher TOC oxidation during stage 3, which resulted in higher IC concentrations in the cultivation medium and, therefore, in higher CO_2 removals by stripping [10].

TN-REs of 16 ± 5%, 19 ± 4% and 22 ± 6% were recorded in stages 1, 2A and 2B, respectively, concomitant with a complete depletion of N-NH_4^+ (Fig. 3b). Nitrification accounted for 65 ± 10%, 69 ± 8% and 58 ± 3% of the influent NH_4^+ during stages 1, 2A and 2B, respectively. Based on this high NH_4^+ oxidation rate, most of TN in the influent as NH_4^+ was recorded in the effluent as NO_3^- and, as it has been above mentioned, the TN-REs were low despite the complete NH_4^+ removal. Thus, TN concentrations in the effluent were similar to N-NO_3^- concentrations from stage 1 to stage 2B, which ranged from 73 ± 5 to 86 ± 6 mg TN L^{-1} (Table 2) and higher than in the influent as a consequence of the evaporative rate (Table 1). Therefore, the effluent would need a further treatment in order to be discharged into the environment. The implementation of this biotechnology at full scale would entail higher

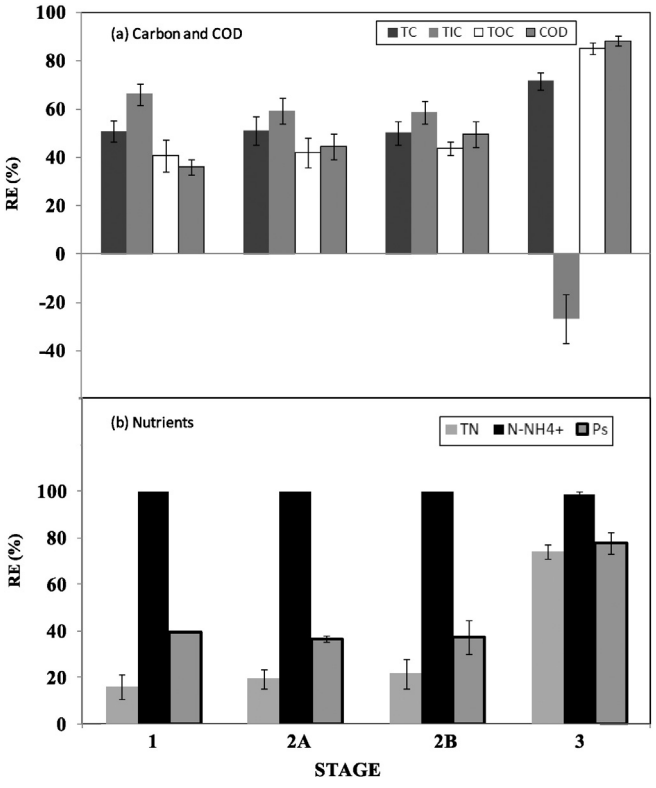

Fig 3. Influence of the operational strategy evaluated on the removal efficiency of a) total carbon (TC), total inorganic carbon (TIC), total organic carbon (TOC) and chemical oxygen demand (COD) and b) total nitrogen (TN), N-NH_4^+ and P_s.

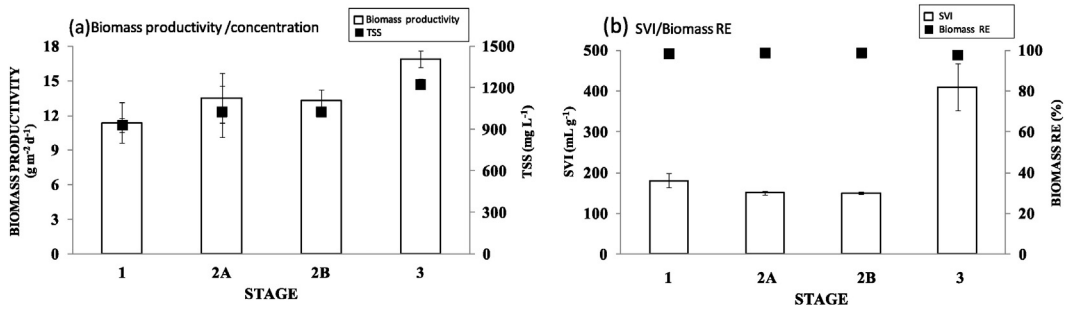

Fig 4. Influence of the operational strategy evaluated on a) biomass productivity (main axis) and biomass concentration in the cultivation broth (secondary axis); and b) SVI (main axis) and biomass removal efficiency in the settler (secondary axis).

biomass productivities as a consequence of the higher light irradiances. In this context, higher biogas flowrates could be supplied to increase the C/N/P ratio in the ADV wastewater (100/26/0.5) which showed carbon limitation compared to the optimum of 100/18/2 [17], and higher nutrient removals would be expected. During stage 3, TN-REs increased to 74 ± 3%, while N-NH_4^+-RE remained at 99 ± 1% and nitrification decreased to 14 ± 3% (estimated from percentage of inlet N-NH_4^+ converted to nitrate), which mediated a significant variation in the TN and N-NO_3^- effluent concentrations (Fig. 3b; Table 2). The fact that similar N-NH_4^+ loading rates, higher IC effluent concentrations in the cultivation medium and DO concentrations above 2 mg O_2 L^{-1} prevailed during stage 3 suggested that the characteristics of the wastewater determined the extent of NH_4^+ nitrification in the HRAP (Tables 1, 2). Nitrogen assimilation into biomass was the only N removal mechanisms from stages 1 to 2B, while only 45 ± 7% of the TN removed was recovered in the harvested biomass during stage 3. Hence, the high nitrification activity in stages 1, 2A and 2B prevented N-NH_4^+ removal by stripping and contributed to TN sequestration in the cultivation medium as N-NO_3^-. These results were in agreement with those reported by García et al. [33] and Posadas et al. [27].

P_s-REs of 39 ± 1%, 36 ± 1% and 37 ± 7% were recorded during stages 1, 2A and 2B (Fig. 3b), respectively, which resulted in average P_s effluent concentrations of 1.2 ± 0.1 mg P_s L^{-1} (Table 2). The P_s-RE increased to 78 ± 5% during stage 3, which decreased effluent P_s concentrations to 0.5 ± 0.1 mg P_s L^{-1}. The higher biodegradability and concentration of organic matter in RV (which modified the C/N/P ratio from 100/26/0.5 in ADV wastewater to 100/11/0.3), likely enhanced P_s removal [17,34]. The phosphorous mass balance calculation conducted confirmed phosphorous assimilation into biomass as the only phosphorus removal mechanism throughout the different operational stages [35].

3.4. Biomass productivity, settleability and elemental composition

Significantly similar biomass productivities of 11.4 ± 1.8, 13.5 ± 2.2 and 13.3 ± 0.9 g m^{-2} d^{-1} were recorded during stages 1, 2A and 2B, respectively, while the higher carbon load during stage 3 induced an increase in biomass productivity up to 16.9 ± 0.7 g m^{-2} d^{-1} (Fig. 4a). Likewise, the TSS concentrations in the cultivation medium were 933 ± 49, 1036 ± 186, 1036 ± 27 and 1228 ± 36 mg L^{-1} during stages 1, 2A, 2B and 3, respectively (Fig. 4a). The above mentioned biomass productivities were similar to those reported in outdoors HRAPs (700–850 L of volume) located in Almería (Spain) (from 4 to 17 g m^{-2} d^{-1}) during the treatment of urban wastewater at 2.8 ± 0.2 d of HRT between July and October [15].

$TSS\text{-}REs_{settler}$ of 98.6 ± 0.5% was recorded regardless to the operational stage at a HRT of 23.5 ± 0.3 min (considering the total liquid external recirculation rate) (Fig. 4b). This efficient TSS recovery by natural biomass settling represented a competitive operational advantage compared to conventional mechanical or chemical methods applied for biomass harvesting [28,36]. The SVI under steady state operation in stages 1, 2A, 2B and 3 accounted for 180 ± 17, 150 ± 6, 150 ± 3 and 410 ± 58 mL g^{-1}, respectively (Fig. 4b). Based on the filamentous nature of the microalgae present in the cultivation medium at all operational stages (see Section 3.5), the different characteristics of RV likely promoted the increase in SVI recorded in stage 3. However, the recorded SVI values were always higher than the 100 mL g^{-1} threshold reported for optimum sludge settling [37].

Similar C, N and P contents of 49.9 ± 0.9%, 8.8 ± 0.1% and 1.2 ± 0.1%, respectively, were recorded in the harvested biomass from stage 1 to 2B. However, the C, N and P content varied to 62.0%, 10.9% and 0.8%, respectively, when RV was fed during stage 3. Despite this variation in biomass composition, the C/N ratio of the harvested biomass remained constant at 5.7 ± 0.1 [12]. These results were in agreement with Posadas et al. [17], who observed different algal–bacterial biomass compositions depending on the initial dilution of the treated agro-industrial wastewaters.

3.5. Microalgae and bacteria population structure

Only the filamentous microalga *Stigeoclonium tenue* present in the inoculum was identified during all operational stages, the rest of the initial microalgae population being replaced along stage 1 (Fig. 5). *Geitlerinema* sp. was the predominant microalga during stages 1, 2A and 3, with an abundance of 63%, 67% and 62%, respectively. The presence of this microalga was not detected during stage 2B, when the filamentous cyanobacterium *Pseudanabaena minima* accounted for 53% of the total number of photosynthetic microorganisms. The high microalgae diversity found in our research was in agreement with that reported in outdoors and indoors pilot HRAPs treating diluted piggery and centrate wastewater [10,38].

The values of Shannon–Wiener diversity index, which takes into account both the sample richness (relative number of DGGE bands)

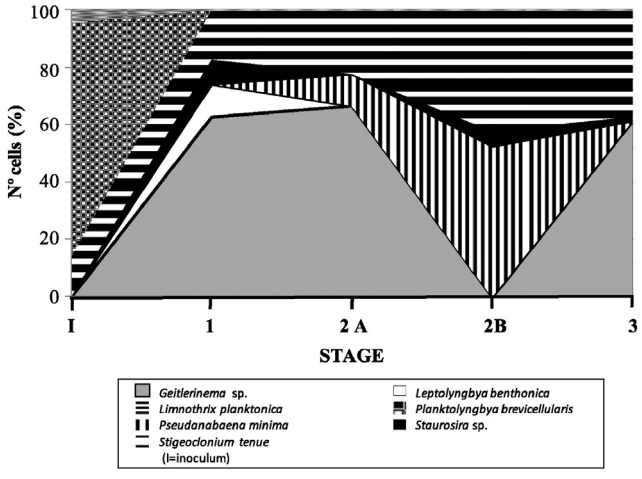

Fig 5. Time course of microalgae population in the HRAP.

and evenness (relative intensity of every band) of the species present in a microbial community, range from 1.5 to 3.5 [39]. In this context, the inoculum exhibited the highest bacterial diversity index (3.3), which remained quite constant during the four operational stages as a result of the rapid microbial acclimation to the varying operational conditions, the lowest bacterial diversity index (2.8) being recorded in stage 2B (Fig. 6).

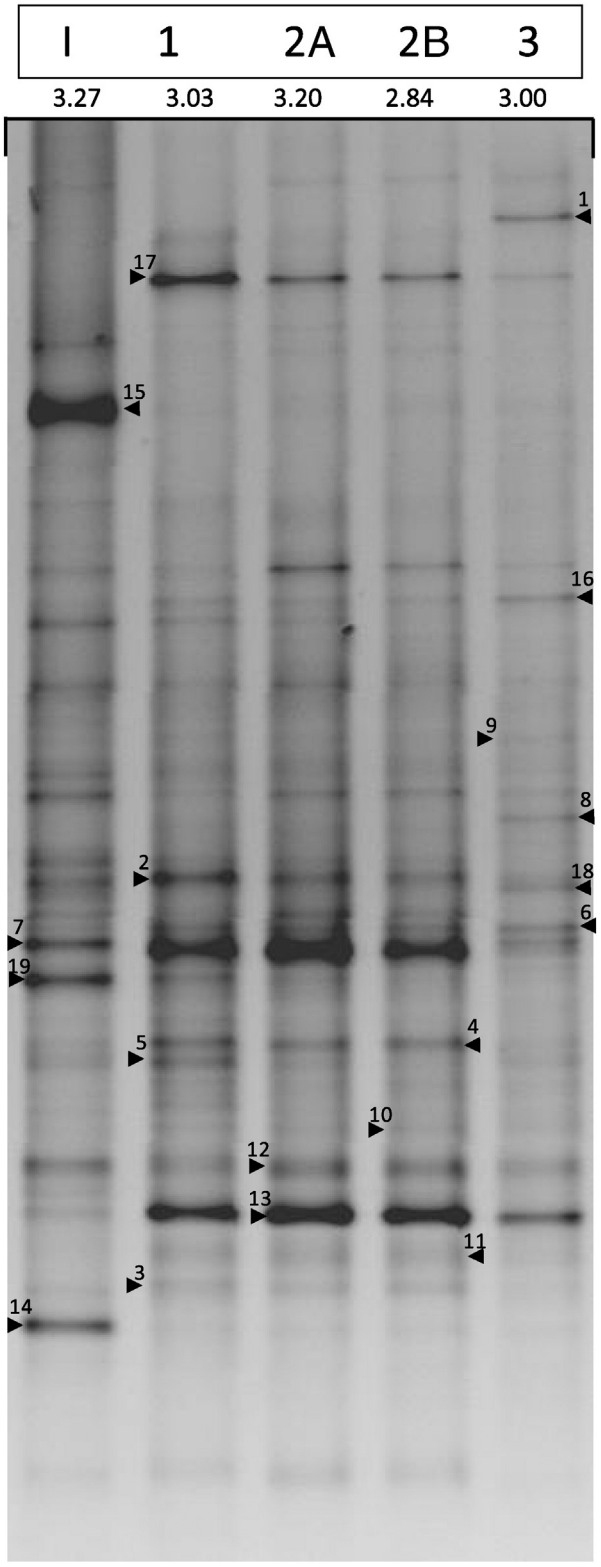

Fig 6. Bacterial DGGE profiles. Sample names and Shannon diversity index are indicated in the upper part of the gel (I = inoculum). The sequenced DGGE bands are indicated with an arrow (black arrow) and the corresponding number of each band.

The analysis of the Pearson similarity coefficients showed low similarities between the inoculum and the microbial communities present in the HRAP from stages 1 (25%) to 3 (19%). The highest Pearson similarity coefficient (91%) was recorded between stages 2A and 2B as a result of their similar operational conditions, while the lowest value (75%) corresponded to stages 1 and 3. From the DGGE gel analysis, 19 bands were sequenced and 5 different phyla were retrieved from the RDP database: Proteobacteria (8 bands), Cyanobacteria/Chloroplast (7 bands), Chlamydiae (2 bands), Firmicutes (1 band) and Chloroflexi (1 band) (Table S1, supplementary material). The phyla Proteobacteria, Cyanobacteria/Chloroplast and Chlamydiae were identified in the inoculum and during the four operational stages (bands 1–17). However, the phylum Chloroflexi was not identified in 2B (band 19) and the phylum Firmicutes (band 18) was only identified in stage 3. The identification of the genus *Catellibacterium* (band 8) and *Simkania* (band 17) and the family *Caldilineaceae* (band 19), which are normally found in wastewaters and in activated sludge processes, supported the efficient aerobic organic matter biodegradation recorded [40–42]. Similarly, the genus *Parachlamydia* (band 16) was also detected during piggery wastewater treatment in an outdoors 465 L HRAP by Ferrerro et al. [43] and its presence has been related to eutrophic aquatic ecosystems and, therefore, to the oxidation of organic matter. Surprisingly, the heterotrophic denitrifying family *Xanthomonadaceae* (band 5) was identified in the inolucum and during stages 1, 2A and 3 despite the aerobic nature of the process [22]. This finding suggested the occurrence of denitrifying activity at the upper part of the absorption column due to a rapid oxygen consumption. This hypothesis could be further supported by the identification of bacteria from the anaerobic genus *Fusibacter* (band 18) during stage 3. The detection of bacteria from the *Alphaproteobacteria* class (bands 6 and 7), which are normally found in nitrification–denitrification processes, correlated with the intense nitrification activity observed along the entire experimentation. The presence of SO_4^{2} in the cultivation medium mediated by H_2S oxidation supported the identification of cyanobacteria (bands 9–13) previously found in terrestrial sulfidic springs [44]. Interestingly, despite the above mentioned low CH_4 solubilization in the recycling cultivation broth, methane oxidizing bacteria belonging to the genus *Methylosarcina* (band 4) were identified in the 4 operational stages [45]. Finally, the absence of aerobic H_2S oxidizing bacteria suggested that H_2S oxidation might occur via chemical rather than biological mechanisms.

4. Conclusions

This research work confirmed the potential of algal–bacterial symbiosis to support a simultaneous wastewater treatment and biogas upgrading. A process configuration based on the direct injection of raw wastewater into the biogas absorption column successfully depleted most of the O_2 present in there cycling cultivation both, which decreased the biomethane O_2 content below permissible levels in most international legislations. Unfortunately, nitrogen desorption resulted in average biomethane N_2 concentrations of 6.3 ± 1.2%, which entailed a decrease in the final biomethane purity. Finally, the fact that aerobic H_2S oxidizing bacteria were not found despite the high microalgae and bacteria diversity present during the entire experimentation suggested that H_2S oxidation might occur via chemical mechanisms.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.algal.2015.09.002.

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SUPPLEMENTARY MATERIAL

Minimization of Biomethane Oxygen Concentration during Biogas Upgrading in Algal-Bacterial Photobioreactors

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CALCULATIONS

a) The CO_2, H_2S and CH_4 removal efficiencies (RE) from biogas at steady state conditions were calculated according to equation (S.1):

$$RE_{CO2/H2S}(\%) = \frac{C_{CO2/H2S/CH4,IN} \cdot F_{IN} - C_{CO2/H2S/CH4,OUT} \cdot F_{OUT}}{C_{CO2/H2S/CH4,IN} \cdot F_{IN}} \cdot 100 \qquad (S.1)$$

where $C_{CO2/H2S/CH4,IN}$ and $C_{CO2/H2S/CH4,OUT}$ stand for the concentrations (%) of CO_2, H_2S or CH_4 in the raw and upgraded biogas in the absorption column, respectively, while F_{IN} and F_{OUT} correspond to the flow rates (L d^{-1}) of the raw and upgraded biogas, respectively.

b) The total carbon (TC) and total inorganic carbon (TIC) removal efficiencies during steady state operation were calculated according to equation (S.2):

$$RE_{TC/TIC} = \frac{(C_{IN} \cdot Q_{IN} + C_{C-CO2,IN} \cdot F_{IN}) - (C_{OUT} \cdot Q_{OUT} + C_{C-CO2,OUT} \cdot F_{OUT})}{(C_{IN} \cdot Q_{IN} + C_{C-CO2,IN} \cdot F_{IN})} \cdot 100 \quad (S.2)$$

where C_{IN} and C_{OUT} stand for the concentration (mg L^{-1}) of total dissolved C (the sum of TOC and IC concentrations for the calculation of TC-RE, and IC concentration for the calculation of TIC-RE) in the influent wastewater and treated effluent , respectively, Q_{IN} and Q_{OUT} correspond to the influent and effluent wastewater flow rates (L d^{-1}) in the HRAP. In this particular calculation, $C_{C-CO2,IN}$ and $C_{C-CO2,OUT}$ were expressed in mg C-CO_2 L^{-1}.

c) The COD, TOC, TN, N-NH_4^+ and P_s removal efficiencies during steady state operation were determined using equation (S.3):

$$RE_i = \frac{C_{i,IN} \cdot Q_{IN} - C_{i,OUT} \cdot Q_{OUT}}{C_{i,IN} \cdot Q_{IN}} \cdot 100 \quad (S.3)$$

where $C_{i,IN}$ and $C_{i,OUT}$ correspond to, respectively, the influent and effluent concentrations (mg L^{-1}) of the target monitored parameter *i* (COD, TOC, TN, N-NH_4^+ or P_s).

d) The total suspended solid removal efficiency of the settler ($RE_{settler}$) was calculated using equation (A.4) during steady state operation:

$$RE_{settler} = \frac{TSS_{HRAP} - TSS_{effluent}}{TSS_{HRAP}} \cdot 100 \quad (S.4)$$

Where TSS_{HRAP} and $TSS_{effluent}$ stand for the TSS concentration (g TSS L^{-1}) in the HRAP and in the effluent, respectively.

d) Biomass productivity (W, g $m^{-2}_{surface\ HRAP}$ d^{-1}) was quantified according to equation (S.5):

$$W = \frac{TSS_{HRAP} \cdot Q_{OUT}}{S} \quad (S.5)$$

where S represents the total HRAP illuminated surface (1.2 m^2).

f) The daily flow rate of harvested biomass ($B_{harvested}$) in the HRAP was calculated according to equation (A.6):

$$B_{harvested} = B_{collected} \cdot \frac{Q_{OUT}}{(Q_{OUT}+Q_{LR})} \cdot 100 \quad (S.6)$$

Where the total collected biomass ($B_{collected}$) in the settler was expressed in L d^{-1} and Q_{LR} represent the external liquid recirculation flow rate (L d^{-1}). The calculation of $B_{harvested}$ in mg d^{-1} was performed through the determination of the humidity and ash content from the $B_{harvested}$ estimated with equation (S.6).

g) The mass balances of C, N and P were determined using equations (S.7), (S.8) and (S.9), respectively:

$$C = (Q_{IN} \cdot C_{IN}) + (C - CO_{2-AC}) - (Q_{OUT} \cdot C_{OUT,}) - (\frac{\%C_{biomass.}}{100} \cdot (TSS_{HRAP} \cdot Q_{OUT} + B_{harvested})) \quad (S.7)$$

$$N = (Q_{IN} \cdot N_{IN}) - (Q_{OUT} \cdot N_{OUT}) - (\frac{\%N_{biomass.}}{100} \cdot (TSS_{HRAP} \cdot Q_{OUT} + B_{harvested})) \quad (S.8)$$

$$P = (Q_{IN} \cdot P_{IN}) - (Q_{OUT} \cdot P_{OUT}) - (\frac{\%P_{biomass.}}{100} \cdot (TSS_{HRAP} \cdot Q_{OUT} + B_{harvested})) \quad (S.9)$$

where C_{IN} , N_{IN} and P_{IN} and $C_{OUT,}$ N_{OUT} and P_{OUT} represent the concentrations of TC (TOC + IC), nitrogen and phosphorus present in the influent wastewater and treated effluent during steady state operation in the HRAP, respectively (mg L^{-1}). C-CO_{2-AC} refers to the total C mass flow rate transferred from synthetic biogas to the liquid phase (mg d^{-1}); % $C_{biomass}$, % $N_{biomass}$ and % $P_{biomass}$ stand for the C, N or P content of the harvested biomass, respectively, with $B_{harvested}$ expressed in mg d^{-1}.

Table S.1. RDP classification of the bacterial DGGE bands sequenced with at least 50% of confidence level, and corresponding closest relatives in Genbank obtained by the BLAST search tool with their similarity percentages, and environments from which they were retrieved. I=inoculum

Taxonomic placement (50% confidence level)	Band n°	I	1	2a	2b	3	Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
Phylum *Proteobacteria*	1					x	Uncultured bacterium (EU104244)	97	Activated sludge
Class *Gammaproteobacteria*	2	x	x	x	x	x	Uncultured bacterium (JX27194)	97	Activated sludge in lab-scale reactor with dissolved oxygen above 2.5 mg/l
							Uncultured *Brenneria sp.* (DQ839349)	97	Sludge in pore of carrier used for treating wastewater
Order *Methylococcales*									
Family Methylococcaceae	3	x	x	x	x	x	Uncultured bacterium (HQ330655)	94	Sediment
Genus *Methylosarcina*	4	x	x	x	x	x	*Methylosarcina quisquiliarum* (NR_025040)	93	Landfill soil
Order *Xanthomonadales*									
Family *Xanthomonadaceae*	5	x	x	x		x	Uncultured bacterium (KM293172)	92	Sludge with earthworm
Class *Alphaproteobacteria*	6	x	x	x	x	x	Uncultured bacterium (KP054170)	95	Nitrification and denitrification reactors
	7	x	x	x	x	x	Uncultured bacterium (JN113077)	92	Environmental_sample
Order *Rhodobacterales*									
Family *Rhodobacteraceae*									
Genus *Catellibacterium*	8					x	Uncultured *Alphaproteobacteria* (CU919839)	91	Mesophilic anaerobic digester which treats municipal wastewater sludge
Phylum *Cyanobacteria/Chloroplast*									
Class *Cyanobacteria*	9	x	x	x	x	x	Uncultured bacterium (JX521411)	92	Terrestrial sulfidic spring
	10	x	x	x	x	x	Uncultured bacterium (JX521411)	94	Terrestrial sulfidic spring
	11		x	x	x	x	Uncultured bacterium (JX521411)	96	Terrestrial sulfidic spring
Family *Family IX*									
Genus *GpIX*	12	x	x	x	x	x	Uncultured bacterium (JX521411)	97	Terrestrial sulfidic spring
	13	x	x	x	x	x	Uncultured bacterium (JX521411)	97	Terrestrial sulfidic spring
Genus *GpXII*	14	x	x	x	x	x	*Phormidium uncinatum* (KF770970)	99	Culture collection
							Uncultured *Phormidium sp.* (JN382225)	99	Mat on sandy surface from Guadarrama river
							Phormidium autumnale (JQ769128)	99	Aquatic environment
Class *Chloroplast*									
Family *Chloroplast*									
Genus *Bacillariophyta*	15	x	x	x	x	x	Uncultured bacterium (AY100532)	99	Air sample
							Uncultured *cyanobacterium* (GU983308)	99	Metalliferous peat rhizosphere
							Uncultured bacterium (FJ747120)	98	Air sample at high elevation site
Phylum *Chlamydiae*									
Class *Chlamydiae*									
Order *Chlamydiales*									
Family *Parachlamydiaceae*									
Genus *Parachlamydia*	16	x	x	x	x	x	Uncultured bacterium (JQ055734)	93	Soil
Family *Simkaniaceae*									
Genus *Simkania*	17	x	x	x	x	x	Uncultured bacterium (KM291942)	99	Sludge with earthworm
							Uncultured bacterium (EF208651)	98	Sandy carbonate sediment
							Uncultured bacterium (FN865932)	97	River water

Taxonomic placement (50% confidence level)	Band n°	I	1	2a	2b	3	Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
Phylum *Firmicutes*									
Class *Clostridia*									
Order *Clostridiales*									
Family *Clostridiales_Incertae Sedis XII*									
Genus *Fusibacter*	18					x	Uncultured bacterium (JX223313)	93	Subsurface aquifer sediment
Phylum *Chloroflexi*									
Class *Caldilineae*									
Order *Caldilineales*									
Family *Caldilineaceae*	19	x	x	x		x	Uncultured bacterium (JN391658)	98	Activated sludge in aerobic tank of activated sludge reactor
							Uncultured bacterium (HQ014651)	98	Wastewater treatment plant

Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways

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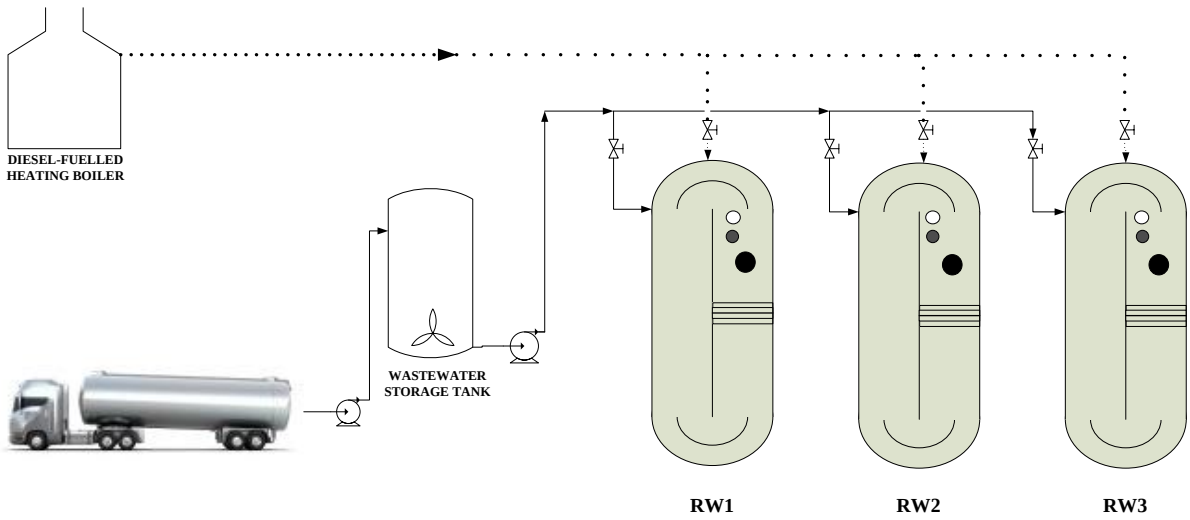
Chapter 8



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Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways

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HIGHLIGHTS

- Effect of pH on wastewater treatment and biomass productivity/composition was null.
- CO_2 from flue gas supported a superior wastewater treatment performance.
- Carbon, nutrients and *E. coli* were efficiently removed from domestic wastewater.
- Maximum biomass productivity of 17 ± 1 $gm^{-2}d^{-1}$ was recorded in the outdoor pilot RWs.

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ABSTRACT

The influence of pH (7, 8 and 9) and CO_2 source (pure CO_2 or CO_2 from flue gas) on both the performance of secondary domestic wastewater treatment and biomass productivity and composition in three outdoors pilot raceways was evaluated for 6 months. Average COD, TN, TP and *Escherichia coli* removal efficiencies of 84 ± 7%, 79 ± 14%, 57 ± 12% and 93 ± 7%, respectively, were recorded. The influence of pH on wastewater treatment was negligible, while the supply of CO_2 from flue gas supported higher COD, TOC and TP removals. Biomass productivities ranged from 4 ± 0 g m^{-2} d^{-1} in December to 17 ± 1 g m^{-2} d^{-1} in July. The highest C, N and P biomass contents (64.8%, 12.6% and 2.4%, respectively) were recorded when flue gas was supplied. Finally, while the protein content in the biomass remained constant (38.2 ± 3.3%), the lipid and carbohydrate contents ranged from 5.8% to 23.0% and from 38.0% to 61.2%, respectively.

1. Introduction

Wastewater management represents an increasing concern worldwide as a result of the exponential human population increase and the rapid industrialization since the mid-20th century. The uncontrolled disposal of domestic and industrial wastewaters into the environment causes severe pollution problems such as eutrophication or oxygen depletion in lakes and rivers, which makes wastewater treatment mandatory [1]. Unfortunately, conventional wastewater treatment technologies present some techno-economic limitations [2]. For instance, process aeration represents 45–75% of the total operation costs in an activated sludge wastewater treatment plant (WWTP) [3], while anaerobic digestion entails a poor nutrient removal [4]. In this context, microalgal-bacterial processes constitute a sustainable and cost-effective alternative to conventional technologies due to their free oxygenation potential and efficient nutrient removal [5]. This green biotechnology is characterized by the oxidation of the organic pollutants present in the wastewater to CO_2 by heterotrophs and by the assimilation of nutrients as a valuable algal-bacterial biomass, which can be further used as a biofertilizer and/or as a feedstock for biofuel production [6,7]. As a result of CO_2 fixation in the presence of light, microalgae photosynthetically provide the O_2 needed by heterotrophs and nitrifiers for the oxidation of organic pollutants and NH_4^+ [8].

Microalgae-based processes were first implemented in the mid 1950s in California for domestic wastewater treatment in algal ponds called raceways (RWs) [9]. RWs are currently the most economic photobioreactor configuration for microalgae cultivation, despite their lower algal biomass productivities when compared to closed photobioreactors [10]. RWs consist of shallow ponds

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(0.1–0.4 m deep) divided into two or four water channels in order to allow liquid mixing and circulation, which is often provided by paddlewheel mechanical agitation [11]. Since their early applications, RWs have supported a cost-effective organic matter and nutrient removal from domestic, industrial and livestock wastewaters [2,12,13]. However, the low C/N/P ratio in most wastewaters, compared to the algal-bacterial biomass composition ratio (≈100/18/2), often limits the efficiency of nutrient removal in microalgae-based wastewater treatment processes due to a carbon deficiency [9,14,15]. In this regard, an external CO_2 addition into the mixed liquor could enhance algal-bacterial biomass productivities and consequently the recovery of nutrients from wastewaters [16]. CO_2 addition would also prevent the rise in pH in the mixed liquor of the RWs mediated by photosynthetic activity, and therefore mitigate nitrogen losses by N-NH_3 stripping and phosphorus precipitation [1,17]. However, despite the potential of this synergistic process integration, the number of outdoors studies assessing at semi-industrial scale the performance of wastewater treatment supported by CO_2 addition is scarce, with the few studies available mainly focused on tertiary wastewater treatment [14,16].

The present work assessed the performance of three outdoors semi-industrial RWs operated in parallel during secondary domestic wastewater treatment for 6 months (July–December) at three different pHs (7, 8 and 9) controlled by the addition of pure CO_2 and CO_2 from real flue gas. The operation of the RWs was also monitored without pH control in order to evaluate the reproducibility of process performance and to serve as control.

2. Materials and methods

2.1. Microorganisms

The RWs were inoculated with *Scenedesmus* sp. previously cultivated in an outdoors thin layer RW and with activated sludge from the WWTP of El Ejido (Almería, Spain) at total suspended solid (TSS) concentrations of 2500 and 4500 mg L^{-1}, respectively. Under the particular environmental conditions of Almería, *Scenedesmus* has been consistently shown as the dominant microalga species, which supports the selection of this microalga for the inoculation of our raceways [10]. In addition, *Scenedesmus* has been also consistently reported as a microalga species commonly found in photobioreactors treating domestic wastewater [18] (Photograph 2a, Supplementary material).

2.2. Experimental set-up

Experiments were conducted in three outdoor raceways (RW1, RW2 and RW3) located at Estación Experimental Las Palmerillas, property of Fundación CAJAMAR (Almería, Spain) (Fig. 1a; Photograph 2b and 2c in Supplementary data). RW1, RW2 and RW3 consisted of three polypropylene algal ponds of two 6-m length channels, 0.6-m width connected by 180° bends at each end, with 8.33 m^2 of illuminated surface and 10 cm of depth. Guide vanes made of polypropylene were placed in the bends of the photobioreactors. The total working volume in RW1, RW2 and RW3 was 700,

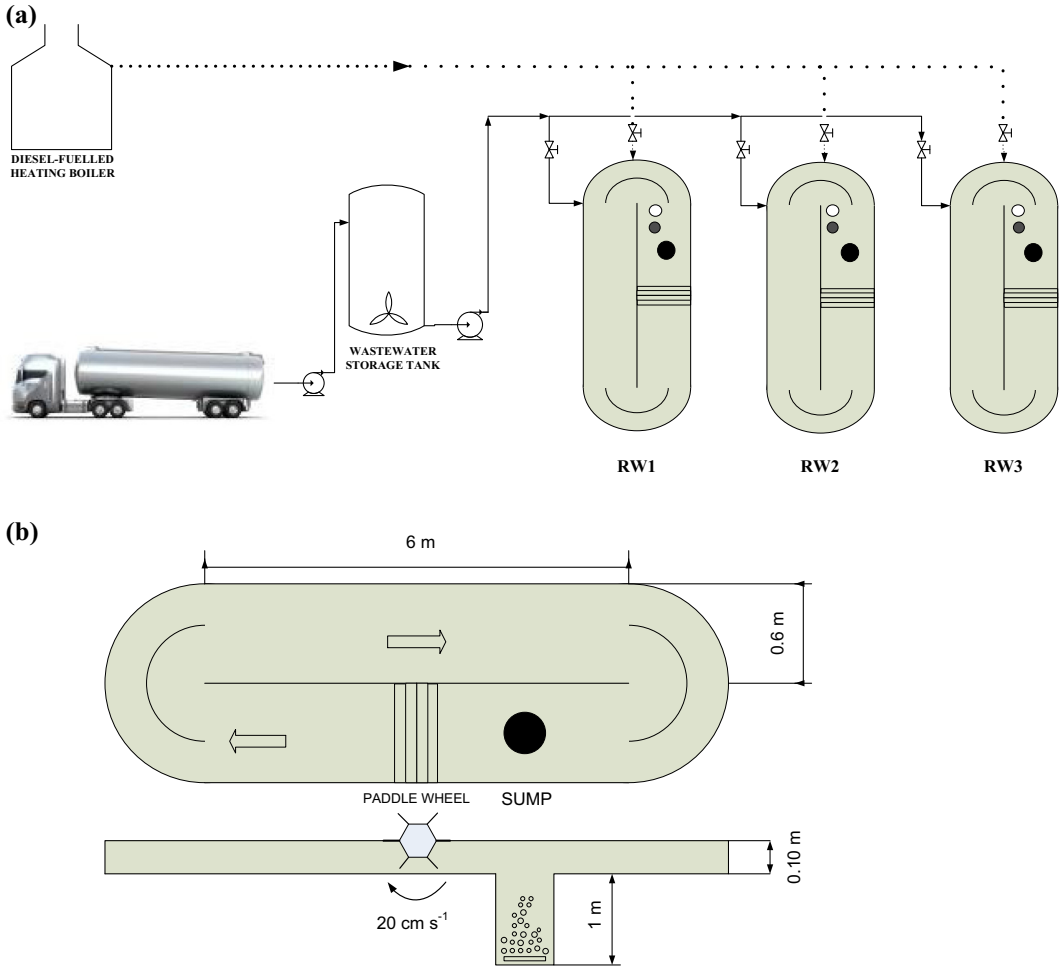

Fig. 1. (a) Schematic of the three raceway photobioreactors. White circles in the RWs represent pH sensor, while grey circles refer to the sensors of dissolved oxygen, temperature, and CO_2 composition. Continuous and discontinuous lines indicate domestic wastewater and CO_2 distribution, respectively. (b) Schematic of a raceway with common dimensions, paddlewheel and sump (black circle).

800 and 850 L, respectively. The main difference in the photobioreactor volume was the setting of a 1-m depth sump to improve CO_2 mass transfer in RW2 and RW3 (Fig. 1b) [19]. The sump volumes in RW2 and RW3 were respectively 100 and 150 L. Culture mixing in the RWs was provided by a six bladed paddlewheel driven by an electric motor (Motovario, Italy), which supported a liquid recirculation velocity of 20 cm s^{-1}.

2.3. Operational conditions

RW1, RW2 and RW3 were initially filled with tap water, and inoculated with *Scenedesmus* sp. and activated sludge at 30% and 10% of their total working volume, respectively. RW1, RW2 and RW3 were initially fed in semi-continuous mode during the first month of operation between 9 and 12 a.m. with primary domestic wastewater using a S-561 82 Husqvarna AB pump (Sweden) at a hydraulic retention time (HRT) of 3.3 ± 0.2 d, and maintained at a pH of 8 (automatically controlled via pure CO_2 sparging) in order to acclimate the microorganisms to cultivation in wastewater. Automatically controlled valves (Cepex, L10, Spain) for wastewater flow rate control were installed after this acclimation period (≈34 days) in order to feed the wastewater into the RWs for 12 h a day. Four operational stages were tested, whose main operational characteristics and objectives are showed in Table 1. Pure CO_2 (stage I) or CO_2 from flue gas (stages II and III) were supplied at the bottom of the sumps (or at the bottom of RW1) through a 25 mm diameter polyethylene diffuser to control the pH. The pH control set points in RW1, RW2 and RW3 were 9, 8 and 7 during stages I and II (operated at a HRT of 2.7 ± 0.1 and 2.8 ± 0.2 d, respectively), while pH 8 was the set point in the three RWs during stage III (operated at a HRT of 6.7 ± 0.4 d) (Table 1). The pH set point of 9 was established in RW1 due to the lower CO_2 mass transfer efficiencies previously recorded in RWs without sump [11]. CO_2 and flue gas supply was regulated by a solenoid on/off valve automatically opened when pH increased over the set point. Air was continuously sparged into the systems in the absence of CO_2 supply to avoid O_2 accumulation into the raceways, and therefore microalgae inhibition due to photo-oxidation [20]. The CO_2, flue gas and air flow rate sparged into the reactors was maintained at 20 L min^{-1} via a mass flow controllers (PF 725S-F01-F, SMC, Tokyo, Japan). Stage IV involved process operation at a HRT of 6.0 ± 0.3 d in the absence of pH control. Operational conditions in each stage were maintained until a steady state was reached (constant values of TSS, maximum quantum yield (F_v/F_m) and culture absorbances at 680 nm).

The parameters monitored on line and logged every 6 min in the RWs were the pH, temperature and dissolved oxygen (DO) concentration in the mixed liquor, the composition of CO_2 in the exhaust flue gas, the duration of CO_2 valve opening (FG line, Italy) and the impinging irradiation at the RWs surface. pH was measured by pH probes (Crison, Spain), temperature and DO in the medium were determined with In Pro 6050/120 oxygen sensors (Mettler Toledo; Spain) and the impinging irradiation with a pyranometer G-54 (LI-COR, USA). The composition of CO_2 in the sparged flue gas at the surface of the raceways was measured using GMM 220 carbon dioxide sensors (Vaisala, Finland) coupled to a fume hood. This measurement took place only when using flue gas since the outlet gas when pure CO_2 was sparged for pH control contained CO_2 concentrations above the maximum concentration measured by GMM 220 (20%). The wastewater influent flow rate was recorded every day using a flow meter (Cepex, Spain), while the effluent flowrate was estimated considering the water evaporation losses in the RWs, which were recorded in a local meteorological station at "Estación Experimental Las Palmerillas". The number of daily sun hours was obtained from on-site solar irradiation measurements, while the average external temperature was also recorded at the local meteorological station of "Estación Experimental Las Palmerillas".

2.4. Primary domestic wastewater and CO_2 sources

Primary domestic wastewater was twice per week transported from El Ejido WWTP to the experimental facility (Las Palmerillas, Almería, Spain) and stored in a feed tank of 5000 L, where it was periodically stirred in order to avoid suspended solids deposition. The wastewater chemical oxygen demand (COD) variations during storage were below 15%. Primary domestic wastewater was subjected to the typical variations of the receiving wastewater in a WWTP in terms of COD, total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), N-NH_4^+, N-NO_3^-, total phosphorus (TP) and *Escherichia coli* (Table 2). Pure CO_2 (99.9%) was purchased from Abello Linde (Spain), while flue gas (10% CO_2) was obtained on-site from a diesel heating boiler (Tradesa, MOD SF 20, RA GTI, TRADE, Italy).

2.5. Sampling procedure and calculations

Liquid samples of 200 mL were daily drawn from the mixed liquor of the RWs to determine the TSS concentration, F_v/F_m and culture absorbance at 680 nm. The characterization of the steady states (during operation at constant TSS concentrations, F_v/F_m and culture absorbances at 680 nm) in each RW was carried out during two consecutive sampling days. F_v/F_m was measured with an Aquapen AP 100 fluorometer (Photon Systems Instruments; Czech Republic), while culture absorbance was determined in a double beam Helios spectrophotometer (Spain). The 200 mL mixed liquor samples were filtered through 0.20 μm (Millipore, Spain) in order to simulate biomass harvesting from the RWs with a membrane module. The concentration of COD, total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), N-NH_4^+, N-NO_3^- and total phosphorus (TP) were determined in the above mentioned filtered cultivation medium of each RW and in 100 mL

Table 1
Main operational parameters and objectives in the tested RWs during the four different operational stages.

Stage	Sampling month	Elapsed time (d)	HRT (d)	pH control	CO_2	Objective
I	August–September	29	2.7 ± 0.1	RW1 = 9; RW2 = 8; RW3 = 7	Pure CO_2	Influence of pH on wastewater treatment, and productivity and composition of the biomass
II	September–October	18	2.8 ± 0.2	RW1 = 9; RW2 = 8; RW3 = 7	CO_2 from flue gas	Influence of the source of CO_2 on wastewater treatment, and productivity and composition of the biomass/ determination of carbon consumption in the raceways at different pHs
III	October–November	32	6.7 ± 0.4	8	CO_2 from flue gas	Influence of the absence of CO_2 addition on wastewater treatment, and productivity and composition of the biomass
IV	December	18	6.0 ± 0.3	Without pH control	No CO_2 addition	

Table 2
Average composition of the primary domestic wastewater supplied to the raceways and of the filtered effluent of the RWs under steady state operation in the four experimental stages.

Stage	Stream	COD (mg L^{-1})	TOC (mg L^{-1})	IC (mg L^{-1})	TN (mg L^{-1})	N-NH_4^+ (mg L^{-1})	N-NO_3^- (mg L^{-1})	P-PO_4^{3-} (mg L^{-1})	*E. coli* (MPN 100 mL^{-1})
I	Influent	575 ± 84	211 ± 5	117 ± 1	64 ± 15	63 ± 14	1 ± 1	9 ± 3	(3 ± 1)·10^6
	RW1	81 ± 1	72 ± 0	50 ± 3	23 ± 1	1 ± 1	22 ± 1	6 ± 1	(30 ± 4)·10^4
	RW2	75 ± 2	65 ± 0	77 ± 1	20 ± 1	1 ± 1	19 ± 3	6 ± 0	(16 ± 10)·10^4
	RW3	116 ± 2	76 ± 0	61 ± 2	25 ± 2	1 ± 1	24 ± 1	6 ± 0	(67 ± 21)·10^4
II	Influent	744 ± 82	313 ± 2	140 ± 6	52 ± 4	50 ± 3	1 ± 1	11 ± 2	(7 ± 0.6)·10^6
	RW1	75 ± 6	57 ± 4	69 ± 3	24 ± 0	4 ± 3	19 ± 0	5 ± 1	(23 ± 0)·10^4
	RW2	103 ± 9	65 ± 5	78 ± 0	15 ± 1	2 ± 1	12 ± 1	5 ± 1	(24 ± 5)·10^4
	RW3	66 ± 4	61 ± 1	73 ± 11	22 ± 3	4 ± 3	18 ± 1	4 ± 2	(70 ± 14)·10^4
III	Influent	649 ± 52	244 ± 4	134 ± 1	75 ± 2	74 ± 1	0 ± 0	10 ± 1	(7 ± 2.0)·10^6
	RW1	106 ± 5	85 ± 8	84 ± 3	16 ± 1	16 ± 1	0	4 ± 0	(23 ± 15)·10^4
	RW2	105 ± 3	77 ± 5	68 ± 4	7 ± 1	6 ± 2	0	4 ± 0	(176 ± 5)·10^4
	RW3	91 ± 2	74 ± 2	73 ± 3	17 ± 3	17 ± 2	0	3 ± 0	(14 ± 14)·10^4
IV	Influent	432 ± 77	181 ± 9	132 ± 23	70 ± 6	66 ± 5	0 ± 0	9 ± 0	(4 ± 1.0)·10^6
	RW1	124 ± 1	76 ± 6	82 ± 12	2 ± 0	2 ± 0	0	4 ± 0	(3 ± 1)·10^4
	RW2	95 ± 8	83 ± 10	73 ± 3	2 ± 0	2 ± 0	0	4 ± 0	(2 ± 1)·10^4
	RW3	148 ± 1	81 ± 10	81 ± 12	2 ± 0	2 ± 0	0	4 ± 0	(5 ± 2)·10^4

MPN: most probable number; *E. coli* = *Escherichia coli*.

liquid samples of primary domestic wastewater drawn from the stirred storage tank. Similarly, 2 mL of the primary domestic wastewater and of the mixed liquor from each RW were seeded into Petri dishes to determine the total concentration of *E. coli* (*Mc. Conkey* nutrient agar, Scharlau, Spain). The absorption in the visible range (400–800 nm) to determine the biomass extinction coefficient (K_a) was also measured during steady state using 3 mL of mixed liquor samples. Biomass was harvested by centrifugation (Digicen 20, Ortoalresa, Spain) for 5 min at 5000 rpm, resuspended in de-ionized water and centrifuged again in order to wash salts prior to lyophilization (Cuddon, New Zealand). The elemental (C, N and P) and macroscopic (lipids, proteins, carbohydrates and ashes) biomass compositions were also determined at each steady state.

Process performance was characterized by the steady state removal efficiency (RE) of COD, TOC, TC, TN, N-NH_4^+, TP and *E. coli*, the mass of CO_2 transferred ($C_{transferred}$) from the gas to the liquid phase, the mass balances of C, N and P, the areal biomass productivity (W) and the biomass extinction coefficient (K_a). The calculation procedures for the above referred parameters are detailed in the Supplementary materials section.

2.6. Analytical procedures

COD, TC (TOC + IC) and TN concentrations were determined using Hack-Lange (Germany) kits (LCI 400, LCKI 381 and 238, respectively). TSS, N-NH_4^+, N-NO_3^- and TP concentrations were determined according to the standard methods approved by the Spanish Minister of Agriculture [21]. *E. coli* was determined according to UNE-EN-ISO 9308-1:2001 [22]. The determination of the C and N content of the algal-bacterial biomass was performed using a LECO CHNS-932 analyzer according to the internal procedure of the University of Almería, while phosphorus content analysis was carried out spectrophotometrically after acid digestion in a microwave according to the internal procedure of the Instrumental Technical Laboratory of the University of Valladolid. Lipids were determined gravimetrically from an extract obtained with 10 mL of a solvent mixture of chloroform:methanol (2:1) (v/v) and 100 mg of dry biomass [23]. The protein content was determined using the Lowry method in dry biomass aliquots of 20 mg [24]. Carbohydrate composition was estimated by the difference between lipids and proteins in the biomass [25]. Finally, total ash content was determined by incineration at 570 °C for 5 h using 0.5 g sample in an oven (Forns Hobersal, Spain).

3. Results and discussion

3.1. Daily fluctuations of environmental parameters and CO_2 addition in the raceways

The daily temperature and DO variations in the RWs were correlated with the diurnal solar radiation cycle, regardless of the raceway configuration and operational conditions (Figs. 2 and 3). The average light irradiation, ambient temperature, number of daily sun hours and evaporation rates decreased throughout the four experimental stages from 468 ± 292 to 300 ± 157 μmol m^{-2} s^{-1}, from 23 ± 1 to 13 ± 1 °C, from 11 to 7 h and from 6.4 ± 1.8 to 2.9 ± 1.4 L m^{-2} d^{-1}, respectively (Table 3).These variations will inherently occur in any outdoors experimentation and impact the performance of the HRAPs.

This deterioration in the environmental conditions resulted in significant decrease in the average temperatures in the mixed liquors of RW1, RW2 and RW3 from 22.5 ± 4.6, 23.8 ± 4.4 and 22.3 ± 4.3 °C, respectively, during stage I, to 11.6 ± 3.2, 11.0 ± 3.0 and 9.8 ± 3.0 °C, respectively, during stage IV (Table 3; Figs. 2 and 3). Despite optimum temperatures for microalgae growth often range from 20 to 30 °C, the successful carbon and nutrient removals from piggery and urban wastewaters recorded in similar RWs of 470 L at average mixed liquor temperatures of 7 and 11 °C, respectively, suggests that the low temperatures recorded during this experimentation in stages III and IV would still allow an efficient microalgae-bacterial wastewater treatment [2,12]. Similarly, maximum DO saturation concentrations of 330%, 197% and 234% were recorded during stage I in RW1, RW2 and RW3, respectively, while the minimum DO saturation concentrations (26%, 85% and 61%, respectively) were recorded during stage IV at night (Fig. 2 and 3). Thus, an O_2-mediated microalgae inhibition could have occurred in stage I in the raceways during peak radiation hours [20], while aerobic conditions always prevailed during the night in all RWs regardless of the operational stage [26]. It is worth noticing that both the highest and lowest DO concentrations were observed in RW1 likely due to the low volumetric mass transfer coefficients in this RW derived from the absence of sump [11].

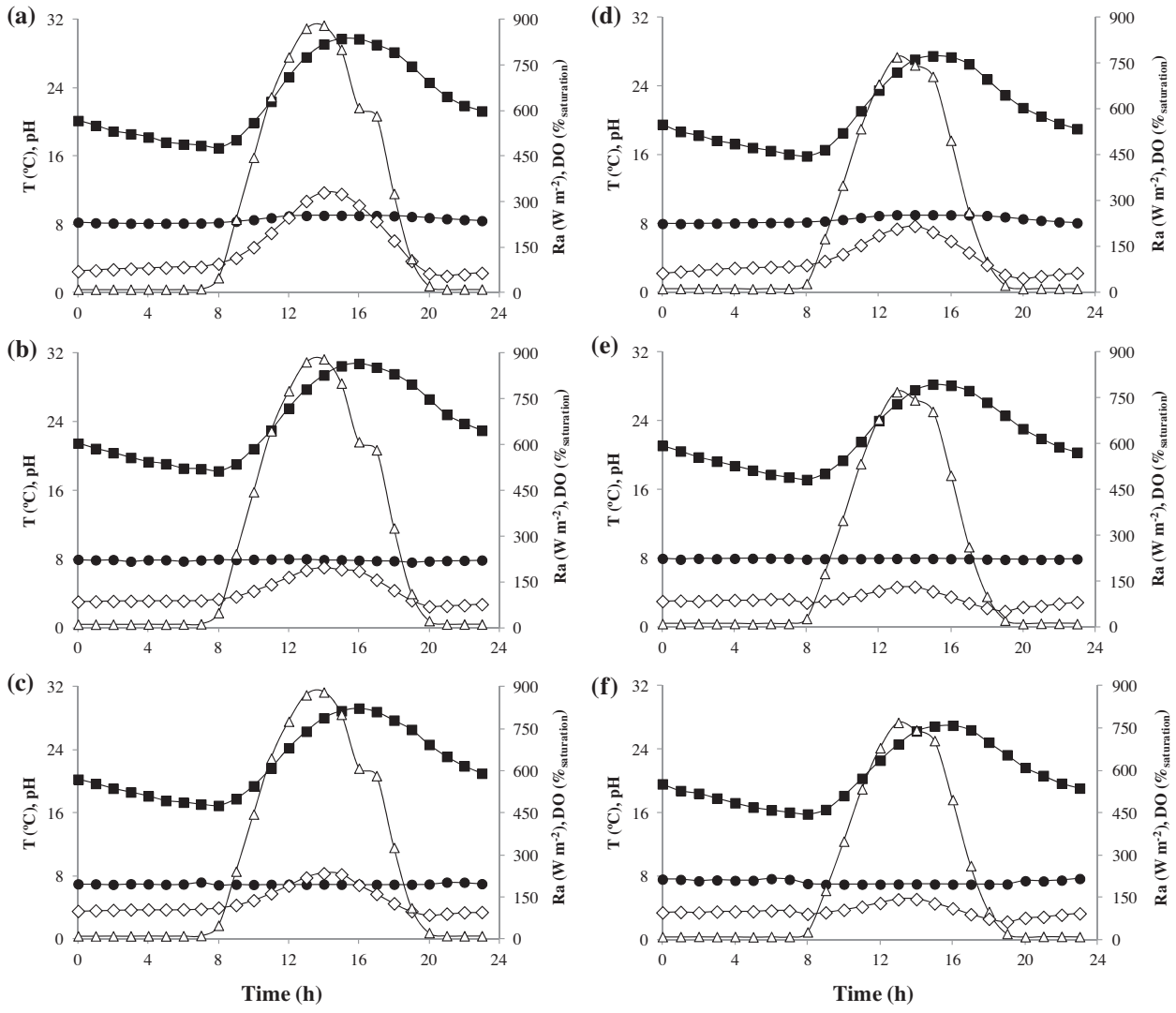

Fig. 2. Daily time course of DO (—◇—), temperature (—■—), pH (—●—) and light radiation (Ra) (—△—) during stage I in RW1 (a), RW2 (b) and RW3 (c), and stage II in RW1(d), RW2 (e) and RW3 (f) under steady state operation.

Likewise, during stage I pH was successfully controlled via pure CO_2 addition at 8.6 ± 0.4, 7.9 ± 0.1 and 7.0 ± 0.1 in RW1, RW2 and RW3, respectively (Fig. 2). Similarly, pH was efficiently controlled via flue gas addition in RW1 and RW2 during stage II (8.4 ± 0.4 and 7.9 ± 0.1, respectively), while flue gas sparging at 20 L min^{-1} was not enough to maintain pH values below 7 during the peak radiation hours in RW3 (7.3 ± 0.3) (Fig. 2f). Under these conditions, the total CO_2 mass transfer rates to the RWs were 5.5, 35.7 and 204.2 mg L^{-1} d^{-1}, which corresponded to CO_2 mass transfer efficiencies to the mixed liquor in RW1, RW2 and RW3 of 6%, 31% and 58%, respectively. During stage III, pH values were successfully controlled at 8.0 ± 0.1, 7.9 ± 0.1 and 7.9 ± 0.1 via flue gas injection. This required CO_2 transfer rates of 25.5, 29.1 and 28.1 mg L^{-1} d^{-1}, which represented CO_2 mass transfer efficiencies of 8.5%, 52% and 38% in RW1, RW2 and RW3, respectively. Overall, higher CO_2 inputs were required at lower pHs and a lower CO_2 mass transfer efficiency was recorded in the RW without sump (RW1) [11]. Likewise, the low CO_2 mass transfer efficiencies showed that bubble residence times in the RWs were insufficient for complete CO_2 absorption from flue gas with the consequent decarbonation through the water channels. These results were in agreement with those reported by Tredici [27] and De Godos et al. [19]. Finally, despite not being controlled during stage IV, the pH in the mixed liquor of RW1, RW2 and RW3 averaged 8.5 ± 0.4, 8.3 ± 0.3 and 8.4 ± 0.4, respectively, and was correlated to light irradiation conditions (Fig. 3d–f).

3.2. Wastewater treatment

3.2.1. Influence of pHs and CO_2 source

The COD-REs achieved during stage I using pure CO_2 to control the pH in RW1, RW2 and RW3 were, respectively, 88 ± 1, 88 ± 0 and 81 ± 1%, which were comparable to the COD-REs of 91 ± 3%, 88 ± 4% and 92 ± 1% recorded during stage II using CO_2 flue gas (Fig. 4a). The COD effluent concentrations remained lower than 125 mg O_2 L^{-1} regardless of the raceway and operational stage (Table 2), which corresponds to the maximum COD concentration established for wastewater discharge into the environment according to Directive 98/15/CEE [28]. Likewise, TOC-REs during stage I in RW1, RW2 and RW3 were, respectively, 71 ± 0%, 73 ± 0% and 68 ± 0%, which were slightly lower than the TOC-REs of 85 ± 1%, 83 ± 1% and 83 ± 2% achieved during stage II (Fig. 4b). Thus, while the almost negligible differences between COD and TOC-REs at pH 7, 8 and 9 suggest a minor influence of pH on organic matter removal from wastewater, the slightly superior efficiencies when using flue gas instead of pure CO_2 to control the cultivation pH

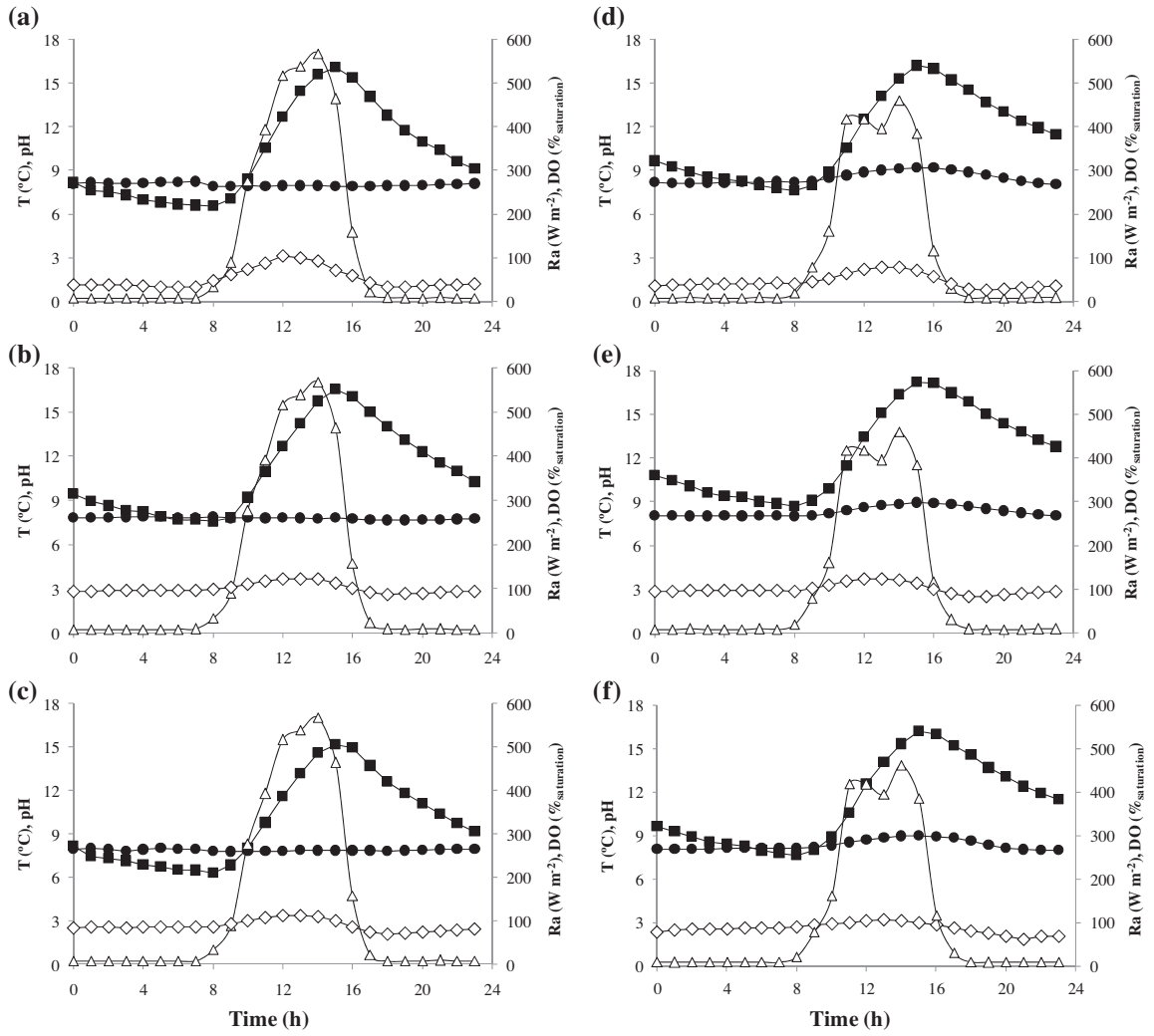

Fig. 3. Daily time course of DO (—◇—), temperature (—■—), pH (—●—) and light radiation (Ra) (—△—) during stage III in RW1 (a), RW2 (b) and RW3 (c), and stage IV in RW1 (d), RW2 (e) and RW3 (f).

Table 3
Average evaporation rate and light irradiance, maximum light irradiance, average outdoors temperature and number of sun hours during the steady state of each experimental stage.

Stage	Evaporation rate ($L\,m^{-2}\,d^{-1}$)	Average light irradiance ($W\,m^{-2}$)	Maximum light irradiance ($W\,m^{-2}$)	Temperature (°C)	N° daily sun hours ($h\,d^{-1}$)
I	6.4 ± 1.8	468 ± 292	881	23 ± 1	11
II	5.2 ± 1.2	462 ± 225	780	22 ± 1	10
III	3.4 ± 1.1	306 ± 216	568	14 ± 3	9
IV	2.9 ± 1.4	300 ± 157	461	13 ± 1	7

showed the advantages of this residual CO_2 source in microalgae-based wastewater treatment [14]. It must be highlighted that the environmental conditions in stage I and II did not vary significantly, which allowed a fair comparison of the influence of the source of CO_2. A carbon mass balance was carried out only in stage II due to the above mentioned technical limitations of the GMT 220 CO_2 analyser to measure high CO_2 concentrations. The mass balance calculation revealed that 39%, 45% and 37% of the total carbon removed from the wastewater and flue gas in RW1, RW2 and RW3, respectively, was recovered in the harvested biomass, with similar IC concentrations regardless of the RW in stages I and II (Table 2).

TN-REs in RW1, RW2 and RW3 accounted, respectively, for 69 ± 2%, 73 ± 1% and 65 ± 1% during stage I, and for 60 ± 6%, 75 ± 3% and 62 ± 6% during stage II (Fig. 4c). This corresponded to specific TN removal rates of 44 ± 6, 41 ± 6 and 48 ± 2 mg TN $g\,TSS^{-1}\,d^{-1}$ during stage I and 28 ± 5, 29 ± 3 and 26 ± 5 mg TN $g\,TSS^{-1}\,d^{-1}$ during stage II, respectively (Table S1, Supplementary data). The maximum concentration of TN permissible for wastewater discharge into the environment according to Directive 98/15/CEE [28] (15 mg N L^{-1}) was achieved only in RW2 during stage II (Table 2). A N mass balance revealed that 81%, 85% and 68% of the TN removed from the wastewater in stage I, and 74%, 61% and 60% during stage II was recovered in the harvested biomass in RW1, RW2 and RW3, respectively. N-NH_4^+-REs in stages I and II were higher than 93% in the three RWs evaluated (Fig. 4d). Despite higher N-NH_4^+ volatilizations would be expected at higher pHs ($NH_3 + H^+ \leftrightarrow NH_4^+$; $pK_a = 9.25$), N-NH_4^+ was rapidly oxidized, which prevented N-NH_4^+ stripping in all RWs [5]. In this context, the high DO and IC concentrations in the mixed liquors, and moderate temperatures, supported an active N-NH_4^+ nitrification, with N-NO_3^- effluent concentrations of 22 ± 1, 19 ± 3 and 24 ± 4 mg N-NO_3^- L^{-1} during stage I, and 19 ± 0, 12 ± 1 and 18 ± 1 mg N-NO_3^- L^{-1} during stage II in RW1, RW2 and RW3, respectively (Table 2). These final N-NO_3^- concentrations corresponded to a significantly similar nitrification activity (estimated as the percentage of influent TN nitrified) during stages I

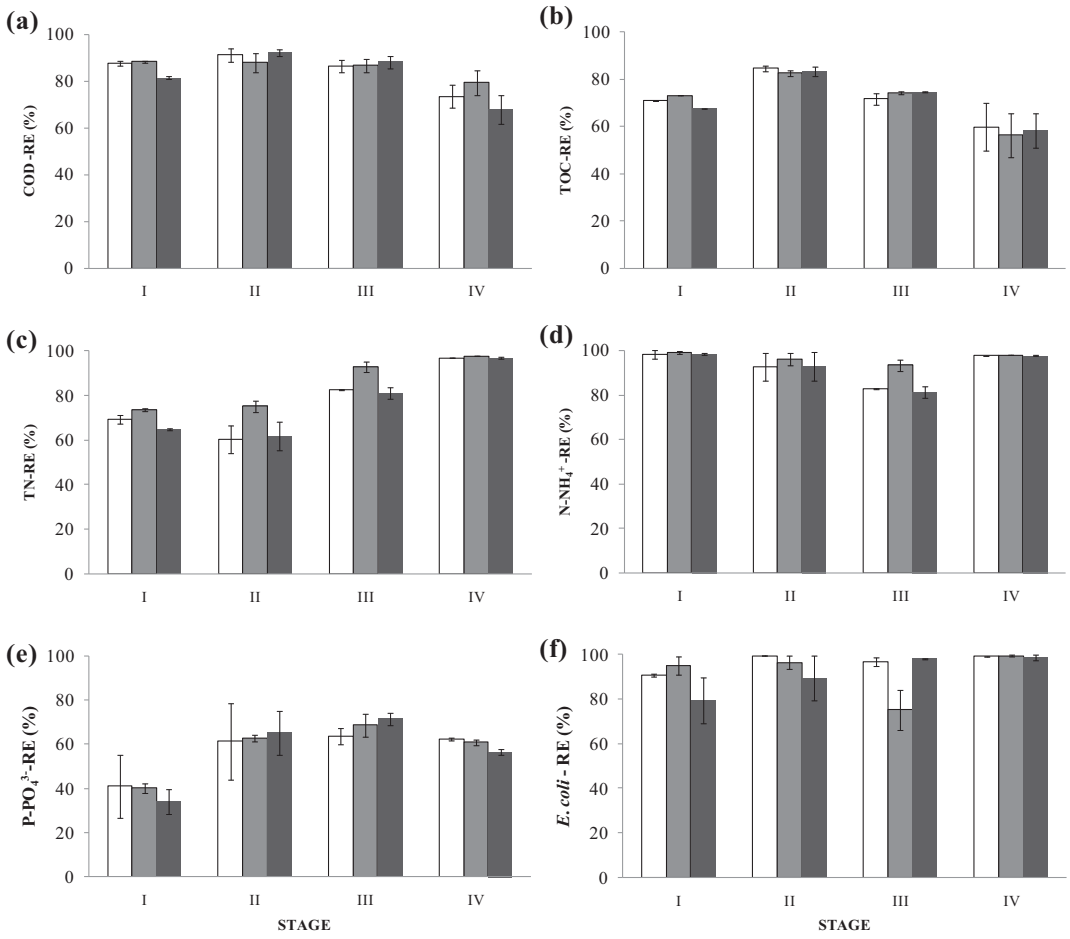

Fig. 4. Removal efficiency of (a) COD, (b) TOC, (c) TN, (d) $N\text{-}NH_4^+$, (e) $P\text{-}PO_4^{3-}$, and (f) *Escherichia coli* in RW1 (□), RW2 (▒) and RW3 (■) during the steady state of the four operational stages.

(30.0 ± 1.2%, 25.7 ± 0.2% and 28.8 ± 8.1%) and II (32.1 ± 0.8%, 21.6 ± 1.0% and 31.2 ± 2.7%) in RW1, RW2 and RW3, respectively. Therefore, neither pH nor the CO_2 source exerted a significant effect on TN-REs. On the other hand, TP-REs in RW1, RW2 and RW3 were, respectively, 41 ± 14%, 40 ± 2% and 34 ± 6% during stage I and 61 ± 17%, 63 ± 2% and 65 ± 10% during stage II, resulting in final TP effluent concentrations of 4–6 mg L^{-1}, which were far above the EU discharge limit of 2 mg L^{-1} [28] (Fig. 4e). This represented specific TP removal rates of 3 ± 1, 3 ± 0 and 3 ± 0 mg TP g TSS^{-1} d^{-1} during stage I and 5 ± 2, 5 ± 1 and 6 ± 0 mg TP g TSS^{-1} d^{-1} during stage II, respectively (Table S1, Supplementary data). In this context, while the pH influence on TP-RE was negligible in the tested range, the use of CO_2 from flue gas exhibited a competitive advantage in terms of TP-RE. Flue gas sparging resulted in lower DO concentrations in the mixed liquors as a result of the higher gas flow rates required to achieve the set pH values (valves were opened for longer periods of time with CO_2 addition from flue gas, data not shown). This could have mediated a higher microalgae and bacterial activity due to the absence of inhibitory DO concentrations and a higher CO_2 availability through the sunny hours in stage II, which likely favored the higher average TOC and TP removals. During stages I and II, 99 ± 1%, 95 ± 4% and 95 ± 2% of the TP removed from the wastewater was recovered in the harvested biomass in RW1, RW2 and RW3, respectively. Therefore, assimilation into biomass was the main phosphorus removal mechanism, even in the RW operated at pH 9 (where $P\text{-}PO_4^{3-}$ precipitation would be expected).

E. coli-REs higher than 80% were recorded in all RWs in stages I and II (Fig. 4f). Higher *E. coli* concentrations were observed when decreasing the pH of the mixed liquor (Table 2), which confirmed the positive effect of high pHs in *E. coli* inactivation [29].

3.2.2. Influence of flue gas addition

Based on the results obtained in stage I and II, the RWs were operated at a constant pH of 8 with flue gas CO_2. The increase in the HRT from 2.8 ± 0.2 (stage II) to 6.7 ± 0.4 (stage III) days did not increase the COD-REs, which accounted for 86 ± 3%, 87 ± 3% and 88 ± 3%, in RW1, RW2 and RW3, respectively. On the other hand, the absence of pH control during stage IV yielded COD-REs of 73 ± 5%, 79 ± 5% and 68 ± 6% in RW1, RW2 and RW3, respectively (Fig. 4a), although this deterioration in the treatment performance was likely due to the less favorable environmental conditions prevailing in stage IV. The similar environmental conditions in stages III and IV allowed for a fair comparison of the influence of pH. COD concentrations in the effluent of the RWs during stages III and IV remained always below the admissible levels for wastewater disposal into the environment, except in RW3 in stage IV (148 mg O_2 L^{-1}) (Table 1). TOC-REs of 72 ± 2%, 74 ± 9% and 75 ± 0% were recorded in stage III in RW1, RW2 and RW3, respectively, which slightly decreased to 60 ± 10%, 56 ± 9% and 58 ± 7% in stage IV (Fig. 4b). These results confirmed the low influence of pH on organic matter removal and the limited process performance of the three RWs during stage IV as a result of the lower DO concentrations in the mixed liquor mediated by the lower irradiances, temperatures and number of daily sun hours (Fig. 3d–f). During stage III, 87%, 67% and 73% of the total carbon removed from the wastewater and flue gas was recovered in the harvested biomass in RW1, RW2 and RW3, respectively. These percentages were con-

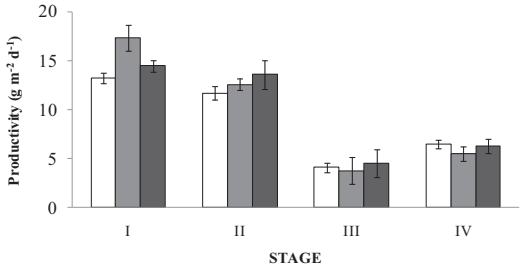

Fig. 5. Biomass productivity in the mixed liquor of RW1 (□), RW2 (▒) and RW3 (■) during the four operational stages.

siderably higher than in stage II. The C mass balance calculation also revealed that the relative contribution of carbon stripping in RW1, RW2 and RW3 was 13, 5 and 10 times lower in stage III than in stage II, as a result of the lower carbon loads supplied at higher HRTs (Table 2). The higher carbon recovery in RW1 during stage III was likely due to the low CO_2 mass transfer efficiency from the flue gas mediated by the absence of sump, which boosted the depletion of the carbon initially present in the wastewater as a result of algal-bacterial biomass growth. During stage IV, 79%, 77% and 84% of the total carbon removed from the wastewater was recovered in the harvested biomass in RW1, RW2 and RW3, respectively. Despite no CO_2 was added to the RWs during stage IV, IC concentrations in the effluent of the RWs remained similar to those recorded in stage III (Table 2).

The increase in HRT in stage III brought about an increase in TN-REs up to 83 ± 0%, 93 ± 2% and 81 ± 3% in RW1, RW2 and RW3, respectively, while operation without pH control in stage IV yielded TN-REs of 97 ± 0%, 98 ± 2% and 97 ± 0%. This corresponded to specific TN removal rates of 21 ± 4, 19 ± 6 and 17 ± 4 mg TN g TSS^{-1} d^{-1} during stage III and 32 ± 8, 37 ± 3 and 33 ± 7 mg TN g TSS^{-1} d^{-1} during stage IV, respectively (Table S1, Supplementary data). This efficient nitrogen removal resulted into final TN concentrations below discharge limits (except in RW1 and RW2 in stage III where TN of 16 and 17 mg L^{-1}, respectively, were recorded) (Fig. 4c, Table 2). The harvested biomass in stage III in RW1, RW2 and RW3, accounted respectively for 48%, 52% and 49% of the TN removed from the wastewaters, while the recovered nitrogen as biomass during stage IV was 35%, 31% and 34%. N-NH_4^+-REs averaged 86 ± 7% during stage III and 98 ± 0% during stage IV in the three RWs (Fig. 4d). The low temperatures prevailing during the last two operational stages likely caused the washout of nitrifying bacteria and consequently no nitrate was detected in these stages. Thus, N-NH_4^+ stripping accounted for most TN removal in the absence of nitrification (stages III and IV), since nitrification contributed to nitrogen sequestration in the previous cultivation stages. These results were in agreement with those reported by García et al. [12], who observed an average contribution of N-NH_4^+ stripping to TN-RE of 32–47% in two HRAPs of 470 L at HRTs of 3–10 d during the treatment of domestic wastewater at outdoor conditions. Similarly, Posadas et al. [5] found a TN-RE decrease from 80% to 60% when nitrification in a 31 L indoor algal turf scrubber photobioreactor treating diluted centrates increased from 9% to 43% at 10.4 ± 0.1 d of HRT. On the other hand, TP-REs during stage III remained at 64 ± 4, 68 ± 5 and 71 ± 3%, and at 62 ± 1, 61 ± 1 and 56 ± 1% in stage IV in RW1, RW2 and RW3, respectively (Fig. 4e). This represented specific TP removal rates of 2 ± 0, 2 ± 0 and 2 ± 0 mg TP g TSS^{-1} d^{-1} during stage III and 3 ± 0, 3 ± 0 and 3 ± 0 mg TP g TSS^{-1} d^{-1} during stage IV, respectively (Table S1, Supplementary data). Despite the superior TP-REs mediated by the increase in HRT, TP effluent concentrations still remained above EU regulatory discharge limits (3–4 mg L^{-1}) during stages III and IV (Table 2). The evaluation of P mass balance revealed that 95 ± 5%, 90 ± 5% and 86 ± 1% of the TP removed from the wastewater in RW1, RW2 and RW3, respectively, was recovered in the harvested biomass in the last two operational stages, which confirmed that assimilation into biomass was the main TP removal mechanism despite the increase in HRT or the absence of pH control.

Finally, *E. coli*-REs during stage III were slightly higher to those recorded during stage II, and accounted for 97%, 75% and 98% in RW1, RW2 and RW3, respectively (Fig. 4f). Stage IV supported the highest *E. coli*-REs (≈99% in the three RWs) among the four stages, which was likely the result of the increase in HRT and the moderately high pH prevailing in the RWs.

3.3. Biomass productivity and characteristics

3.3.1. Influence of pHs and CO_2 source

No influence of the source of CO_2 on biomass productivity was recorded. Hence, areal productivities using pure CO_2 accounted for 13 ± 1, 17 ± 1 and 14 ± 1 g m^{-2} d^{-1} in RW1, RW2 and RW3, respectively, and for 12 ± 1, 13 ± 1 and 14 ± 1 g m^{-2} d^{-1} using flue gas (Fig. 5). These productivities were in agreement with the reported biomass productivity range in outdoors pilot-industrial RWs (10–35 g m^{-2} d^{-1} [30]). However, the TSS concentrations in the three RWs recorded along the four operational stages (321–494 mg L^{-1}) were low compared to those observed in RWs treating agro-industrial wastewater in previous works. For instance, De Godos et al. [2] reported maximum biomass concentrations of 1500 mg TSS L^{-1} in a 464 L RW treating piggery wastewater at 10 d of HRT, pH 8, 15 °C and 167 W m^{-2}. Similarly, Posadas et al. [31] recorded maximum biomass concentrations of 2000 mg TSS L^{-1} in a 180 L RW treating fish farm wastewater diluted with urban wastewater at 7 d of HRT, pH 8.7, 13 °C and 195 W m^{-2}. This showed the high influence of the nature of the treated wastewater on the biomass concentrations in the RW mixed liquor, and consequently on biomass productivity. The slightly higher TSS concentration in RW2 during stages I and II (when environmental conditions remained similar), which also resulted in lower extinction coefficients compared to RW1 and RW3 (0.08–0.12 m^2 g^{-1} compared to 0.15–0.25 m^2 g^{-1}) (Table 4), suggested a favored algal-bacterial biomass growth at pH 8. The quantum yield in stages I and II remained constant at 0.34 ± 0.01 (Table 4), which were low compared to typical reported yields of 0.75 in synthetic mineral salt medium [32] but similar to the quantum yields (0.38) in domestic wastewater [25].

3.3.2. Influence of flue gas addition

The increase in HRT brought about a significant decrease in biomass productivities compared to stage II, which accounted for 4 ± 0, 4 ± 1 and 5 ± 1 g m^{-2} d^{-1} in RW1, RW2 and RW3, respectively. Based on the similar TSS concentrations regardless of the opera-

Table 4
TSS, K_a and F_v/F_m in the mixed liquor of the tested RWs during the steady state in each operational stage.

Stage	RWs	TSS (mg L^{-1})	K_a (m^2 g^{-1})	F_v/F_m
I	RW1	448 ± 81	0.15	0.33 ± 0.03
	RW2	494 ± 11	0.08	0.34 ± 0.03
	RW3	430 ± 74	0.23	0.33 ± 0.03
II	RW1	407 ± 17	0.20	0.32 ± 0.01
	RW2	432 ± 37	0.12	0.35 ± 0.01
	RW3	422 ± 65	0.25	0.35 ± 0.02
III	RW1	396 ± 10	0.15	0.59 ± 0.01
	RW2	403 ± 31	0.16	0.46 ± 0.01
	RW3	427 ± 4	0.20	0.54 ± 0.02
IV	RW1	397 ± 8	0.13	0.50 ± 0.01
	RW2	321 ± 30	0.14	0.47 ± 0.05
	RW3	350 ± 27	0.17	0.43 ± 0.04

Table 5
Carbon, nitrogen, phosphorous, lipid, protein and carbohydrate (expressed on ash free basis) content of the harvested biomass of the RWs under steady state operation.

Stage	RWs	C (%)	N (%)	P (%)	Lipid (%)	Protein (%)	Carbohydrate (%)
I	RW1	42.9	8.7	1.6	6.0	41.3	52.7
	RW2	43.9	8.9	1.2	5.8	40.3	53.9
	RW3	37.5	6.4	1.1	7.2	31.2	61.2
II	RW1	50.4	12.6	2.4	23.0	39.0	38.0
	RW2	61.5	10.1	2.2	18.4	42.9	38.7
	RW3	52.8	9.5	2.0	16.7	42.0	41.3
III	RW1	64.8	10.1	2.3	10.3	38.9	50.8
	RW2	62.3	10.0	2.3	14.9	36.2	48.9
	RW3	61.6	10.0	2.0	15.5	36.3	48.2
IV	RW1	49.9	8.4	1.3	13.9	38.7	47.4
	RW2	51.3	8.5	1.2	14.5	35.2	50.3
	RW3	53.4	9.0	1.3	13.4	35.8	50.8

tional stage and RW (Table 4), it can be concluded that the HRT strongly influenced biomass productivity. On the other hand, process operation in the absence of pH control supported biomass productivities of 7 ± 0, 5 ± 1, 6 ± 1 g m^{-2} d^{-1} during stage IV (Fig. 5). These results clearly showed that CO_2 addition from flue gas did not result in a biomass productivity increase under operation at high HRT. At these operational conditions, the extinction coefficient ranged from 0.13 to 0.20 m^2 g^{-1} (Table 4). These K_a values were relatively low compared to the minimum of 0.19 m^2 g^{-1} recorded when secondary wastewater was treated in 250 mL photobioreactors [25], which was likely induced by the high solid concentration (1700 mg L^{-1}) supported by this particular photobioreactor lab scale configuration. The average quantum yield, F_v/F_m, during stages III and IV were 0.53 ± 0.07 and 0.47 ± 0.04, respectively (Table 4). These higher quantum yields compared to stages I and II at lower irradiances, temperatures and sun hours suggested a possible microalgae activity increase at low irradiances. Similar results were reported by Vonshak and Torzillo [33], who found a reduction of 30% in the quantum yield when irradiance increased from 167 to 750 W m^{-2} at 25 °C in outdoors tubular photobioreactors.

3.4. Biomass composition

3.4.1. Influence of pHs and CO_2 source

A higher C, N and P content was observed in the biomass when flue gas (stage II) was sparged into the mixed liquors regardless of the pH (Table 5). The highest carbon content was recorded at pH 8 (43.9% and 61.5% in stages I and II, respectively) and the lowest N and P contents at pH 7 (N: 6.4% and 9.5% and P: 1.1% and 2.0% during stages I and II, respectively). No influence of the pH on the macromolecular composition (in terms of lipid, protein and carbohydrate content) of the biomass generated along stages I and II was recorded (Table 5). In this context, the protein content remained constant during the four operational stages (≈38 ± 3%), resulting in a constant Protein/N ratio of 4.1 ± 0.5, in agreement with the 4.4 ratio reported by González-López et al. [34]. The biomass lipid and carbohydrates contents exhibited the largest variation with operational conditions. Thus, while the supply of pure CO_2 supported lipid contents of 6.0%, 5.8% and 7.2% in RW1, RW2 and RW3, respectively, CO_2 addition from flue gas unexpectedly increased the lipid content up to 23.0%, 18.4% and 16.7% in RW1, RW2 and RW3, respectively. Conversely, carbohydrate accumulation was favored by the supply of pure CO_2. In this context, lipid synthesis by microalgae cells could have been influenced by the higher CO_2 availability when using flue gas as a result of its more constant supply. Despite the reasonably high biomass productivity and the highest lipid content during stage II, the resulting biodiesel productivities are not profitable for biodiesel production with the current cost of fossil fuels [6].

3.4.2. Influence of flue gas addition

The increase in HRT led to higher C biomass contents in RW1 and RW3 (64.8% and 61.6%, respectively), and in similar N and P content (≈10% N and ≈2% P) (Table 5). The impact of the decrease in temperature from stage II to stage III on C biomass content cannot be however ruled out, but these variations are inherent to outdoors experimentation at semi-industrial scale. On the other hand, process operation in absence of CO_2 from flue gas sparging (stage IV) resulted in a significant decrease in the C, N and P biomass content regardless of the RW (Table 5). These results were in agreement to the empirical C compositions reported by Arbid et al. [16], who respectively recorded a C content increase from 40.2 ± 1.5% and 40.0 ± 1.0% to 43.5 ± 1.8% and 42.5 ± 1.6% in HRAPs with and without sump at 8 d of HRT under outdoors conditions during the treatment of domestic wastewater. Overall, flue gas sparging in stages II and III during wastewater treatment supported the highest carbon biomass content compared to process operation with addition of pure CO_2 or in the absence of pH control (Table 5). Likewise, the highest nitrogen and phosphorus biomass contents were also recorded during the process operation with flue gas addition (II and III) regardless of the pH. The C/N and N/P ratios of the harvested biomass throughout the four operational stages were both 6 ± 1, which highlighted the consistent chemical composition of the algal-bacterial biomass generated despite the changes in operational conditions. The constant C/lipid of 4.3 ± 1 in stages III and IV showed that flue gas sparging did not impact lipid synthesis under these operational conditions. This fact could have been caused by the higher influence of other factors such as lower light irradiances, number of sun hours [35] or temperatures (compared to stages I and II) on lipid synthesis.

4. Conclusions

The influence of pH was negligible in terms of wastewater treatment performance, while CO_2 sparging from flue gas instead of pure CO_2 supported slightly higher COD and TOC-REs, and significantly higher TP-REs. On the other hand, CO_2 addition from flue gas compared to process operation without CO_2 supplementation contributed to pH control but did not improve wastewater treatment performance or biomass productivity as a result of the intensive CO_2 stripping from the RW mixed liquor. Finally, biomass C, N and P content, and macroscopic composition, were significantly impacted (except for protein) by the nature of the supplemented CO_2. Overall, flue gas sparging for pH control was shown the most effective and environmentally friendly alternative for RWs operation due to its contribution to greenhouse gas emission mitigation concomitantly with wastewater treatment and production of a valuable microalgae biomass.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2014.12.059.

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SUPPLEMENTARY DATA

Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways

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Content:

1. CALCULATIONS

a) Removal efficiencies (REs) of COD, TOC, TC, TN, N-NH_4^+, TP and *E. Coli* were calculated under steady state conditions according to equation (1) in each RW:

$$RE = \frac{(Q_{in} \cdot D_{in} - Q_{out} \cdot D_{out})}{Q_{in} \cdot D_{in}} \cdot 100 \quad (1)$$

where Q_{in} represents the influent wastewater flow rate (L d^{-1}) and Q_{out} the effluent flow rate in each RW (L d^{-1}). D_{in} and D_{out} are the influent and effluent concentrations of COD, TOC, TC, TN, N-NH_4^+, TP and *E. Coli*, respectively (in mg L^{-1} or cfu 100 mL^{-1}).

b) The CO_2 transferred from the gas to the cultivation broth (mg L^{-1} d^{-1}) was calculated using equation (2):

$$CO_{2\,transferred} = Q_{gas/air} \cdot t \cdot \frac{(y_{CO2,inlet} - y_{CO2,outlet})}{V_{RW}} \quad (2)$$

where $Q_{gas/air}$ represents the flow rate of flue gas or air sparged in the RW, respectively (mg min^{-1}); t ($min_{gas/air}$ d^{-1}) corresponds to the elapsed time when valves were opened; and $y_{CO2,outlet}$ are the CO_2 gas molar fraction at the inlet and outlet flue gas or air in the RWs, respectively, and V_{RW} is the total working volume of each RW (L).

c) The carbon, nitrogen and phosphorus mass balances expressed in mg d^{-1} were evaluated according to equations (3), (4) and (5):

$$C = (Q_{in} \cdot C_{in,liquid}) + (C - CO2_{transferred}) - \left(Q_{out} \cdot C_{out,liquid}\right) - \left(\frac{\%C_{biomass.}}{100} \cdot TSS_{RW} \cdot Q_{out}\right) \quad (3)$$

$$N = (Q_{in} \cdot N_{in,liquid}) - \left(Q_{out} \cdot N_{out,liquid}\right) - \left(\frac{\%N_{biomass.}}{100} \cdot TSS_{RW} \cdot Q_{out}\right) \quad (4)$$

$$P = (Q_{in} \cdot P_{in,liquid}) - \left(Q_{out} \cdot P_{out,liquid}\right) - \left(\frac{\%P_{biomass.}}{100} \cdot TSS_{RW} \cdot Q_{out}\right) \quad (5)$$

where $C_{in,liquid}$, $N_{in,liquid}$ and $P_{in,liquid}$ and $C_{out,liquid}$, $N_{out,liquid}$ and $P_{out,liquid}$ represent the concentration of total carbon, nitrogen and phosphorus present in the influent wastewater and treated effluent during steady state operation in the RWs, respectively (mg L^{-1}), and C-CO_2 refers to the total C mass transferred from flue gas or air to the liquid phase (mg d^{-1}), respectively; % $C_{biomass}$, % $N_{biomass}$ and % $P_{biomass}$ stand for the C, N or P content in the harvested biomass, respectively, and TSS_{RW} corresponds to the TSS concentration in the cultivation broth (mg L^{-1}).

d) The areal biomass productivity (W) expressed in g m^{-2} d^{-1} was determined according to equation (6):

$$W = \frac{TSS_{RW} \cdot Q_{out}}{S} \quad (6)$$

where S is the illuminated surface in the RWs (8.33 m^2).

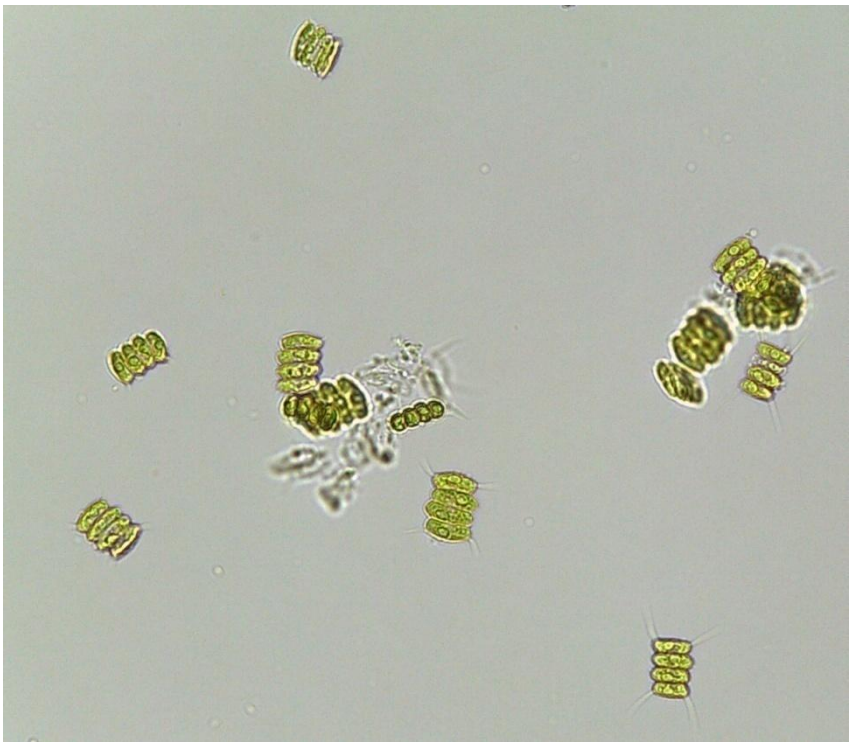
e) The biomass extinction coefficient (K_a) was determined according to equation (7):

$$K_a = \frac{\overline{Abs}}{TSS_{RW} \cdot P} \quad (7)$$

where $\overline{Abs}$ represents the average culture absorbance in the visible spectrum (400-800 nm) and P the light path of the cuvette (m).

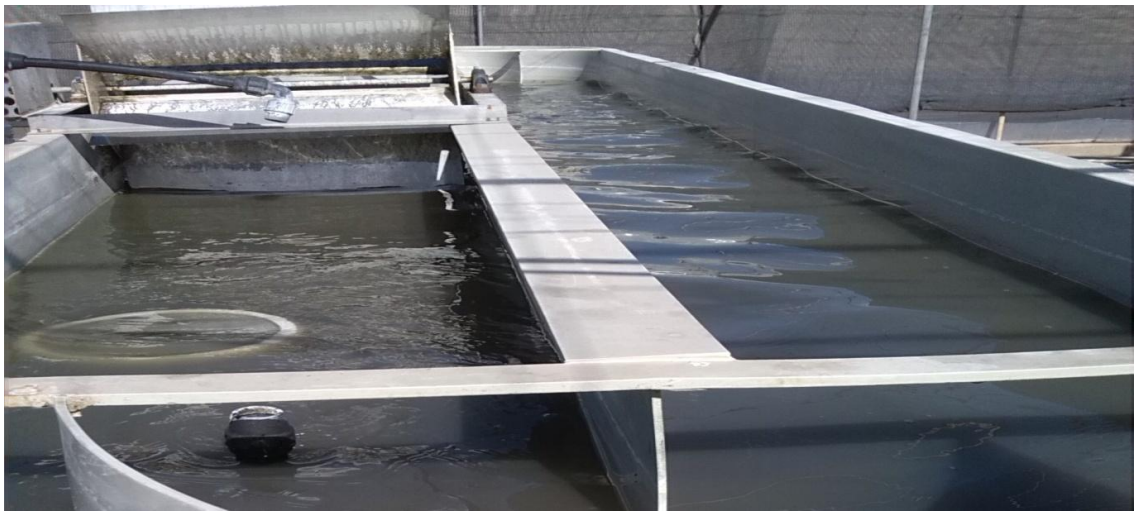
2. PHOTOGRAPHS

Photograph 2a. *Scenedesmus* microscopic view (microalgae used as inoculum)



Photograph 2. Outdoor raceways pilot plants. **b)** RW1 before inoculation and **c)** continuous microalgae cultivation.

b)



c)

Table S1

Specific consumption rates of TN and TP in each RW under steady state operation in the four experimental stages.

Stage	RWs	TN (mg TN $gTSS^{-1}$ d^{-1})	TP (mg TP $gTSS^{-1}d^{-1}$)
I	**RW1**	44±6	3±1
	RW2	41±6	3±0
	RW3	48±2	3±0
II	**RW1**	28±5	5±2
	RW2	29±3	5±1
	RW3	26±5	6±0
III	**RW1**	21±4	2±0
	RW2	19±6	2±0
	RW3	17±4	2±0
IV	**RW1**	32±8	3±0
	RW2	37±3	3±0
	RW3	33±7	3±0

Nutrient removal and solid management restrict the feasibility of algal biofuels generation via wastewater treatment

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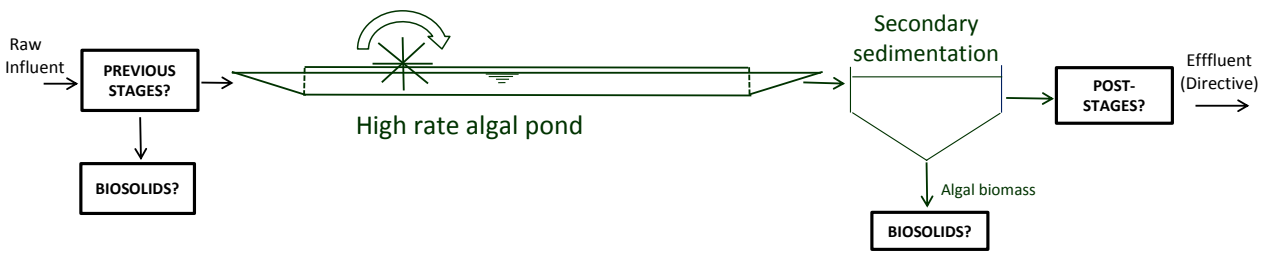
Chapter 9



Nutrient removal and solid management restrict the feasibility of algal biofuel generation via wastewater treatment

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Nutrient removal and solid management restrict the feasibility of algal biofuel generation via wastewater treatment

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ABSTRACT

While numerous studies have demonstrated the efficiency of secondary wastewater treatment in high rate algae ponds (HRAPs), little consideration has been given to how the algal unit should be best integrated within a full treatment system complying with typical nutrient discharge standards. Using the case study of a 2,000 person equivalent (P.E.), we first demonstrate algal treatment is most efficiently used for combined carbon and nutrient removal because an HRAP designed for compliant N (or P) removal *de facto* provides free and environmentally-benign carbon removal. The large O_2 excess capacity for aerobic carbon removal also suggests primary suspended solid removal is unlikely needed (grit removal remains necessary). We then demonstrate combining algal cultivation with anaerobic digestion is not economic at small scale because it offers marginal energy savings (e.g. 10.7 € $P.E.^{-1}$ yr^{-1}) against significant costs for

digestate transport offsite (e.g. 32.5 € P.E.$^{-1}$ y^{-1} at a distance of 50 km). Subsequent sensitivity analyses confirmed that while the potential of algae-to-biogas via WWT is limited (e.g. total cost of 38.2 € P.E.$^{-1}$ y^{-1} in our base case), integrating the use of HRAPs with solar drying provides an economical and energy-efficient WWT alternative for nutrient removal and recovery (24.4 € P.E.$^{-1}$ y^{-1}).

INTRODUCTION

Microalgae biotechnologies are broadly heralded as sustainable platforms for wastewater treatment (WWT) because microalgae provide additional capacities for oxygen supply, nutrient removal, and resource recovery.[1,2] While numerous studies have indeed demonstrated the efficiency of the 'algal unit' for treatment and/or resource recovery via biomass production and valorization (Table 1, Table S1), little consideration has been given to how the algal unit should be best integrated within the full treatment system (Table 1, Table S2). It is however critical to consider the full treatment system when designing and operating a single unit due to synergetic or antagonist effects (e.g. nutrient assimilation into biomass versus sludge management). For example, Steele et al.[3] noticed the importance of minimizing biosolids production during algal WWT due to the high projected costs of biosolid disposal and transport.

The present research explores how HRAPs can be best integrated within a full treatment system capable of efficiently using land, energy and water while meeting stringent requirements for nutrient removal and biosolid management. Emphasis was given to nutrients removal for two reasons: First, it is especially relevant to algal WWT as WW is seen as a source of nutrients for algae cultivation in many 'algae to energy' projects.[1,4] Second, nutrients must be removed from wastewater prior discharge and the

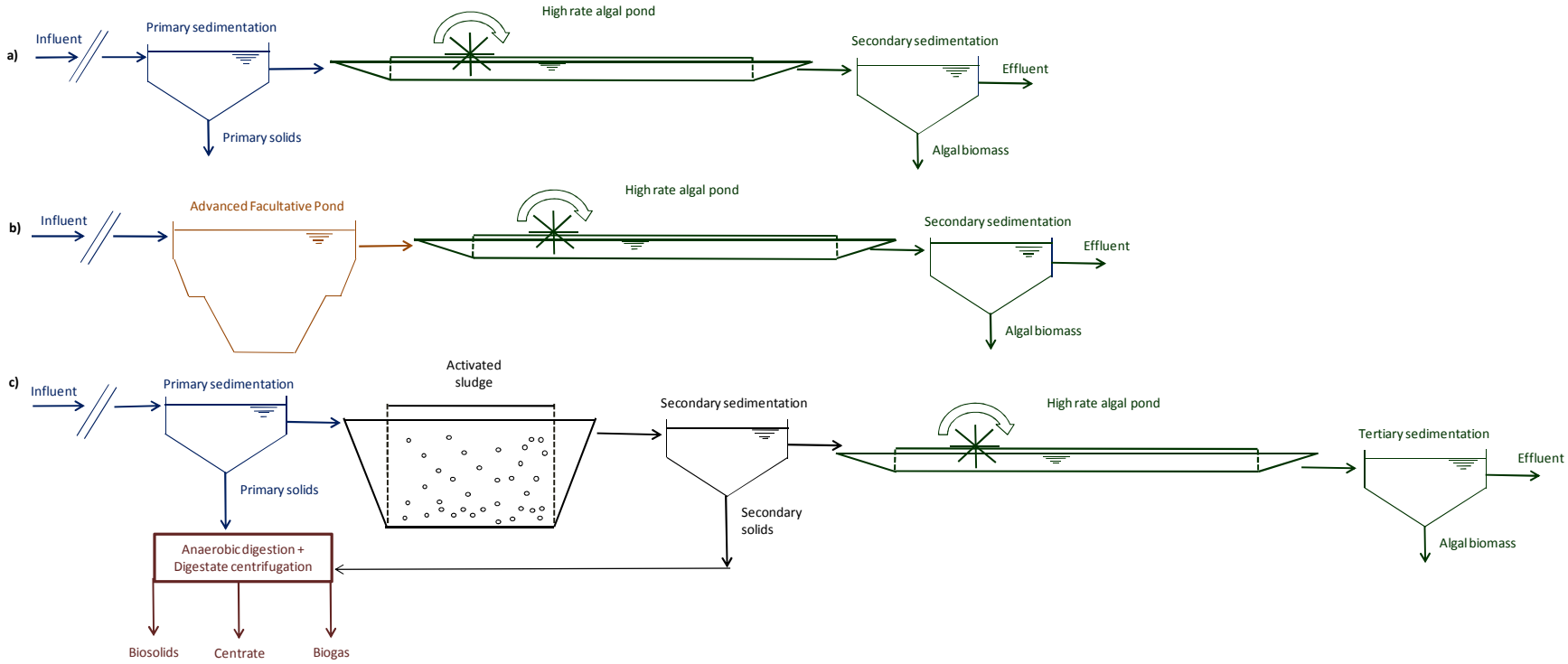
nutrients assimilated within biosolids must be safely disposed of, even following biosolid digestion. The latter is indeed seldom addressed in the literature, where it is often proposed that biosolids or their residues can be used as fertilizers without considering practical limitations.[3,5] The scope of this study was therefore reduced to cases where wastewater land irrigation is not possible due to economic, technical, or regulatory limitations, meaning that nutrients must be removed from the wastewater and disposed safely. As a general strategy, the main algal-based WWT system configurations discussed in the literature were first identified and classified based on the function of the algal unit (Fig. 1, Table 1). Two relevant sub-configurations where the algal-unit is used for complete biodegradable chemical oxygen demand (bCOD) and nitrogen (N) removal where then designed, modeled and compared.

Because algal-based WWT requires large areas of land[6] and wastewater transport over long distances is seldom practical,[7,8] this research hypothesizes algal-based WWT will be most efficiently used for small to medium scale WWT.[3,9] A 2,000 person equivalent (P. E.) community currently treating WW using a low cost system (e.g. stabilization pond) was used as case study, a scenario representative of thousands of communities worldwide.[10] The 2,000 P.E. capacity also represents the smallest WWT size above which nutrient (N, P) discharge is regulated in the European Union (EU).[11] This threshold should not be regarded as a strict, universal, or static limit for the application of HRAPs, the focus being on cases where algal-based WWT is feasible but must comply with nutrient discharge standards. It should finally be noted that this study does not aim to design a 'perfect' universal algal-based WWT because each system must be specifically designed based on local constrains. Instead, this study identifies knowledge gaps in order to improve customized design and identify strategic research areas.

Table 1. Typical algae-based domestic WWT configurations described in published HRAP studies considering biosolid management (the ultimate disposal of digested or processed biomass was however seldom discussed).

Process configuration (Figure 1)	Biosolids treatment/ reuse	Reference
a	Not considered	García et al.[12]
a	Proposal of use for biofuel production	Park et al.[13]
a	Proposal of use for anaerobic digestion	Craggs et al.[2]
a	Proposal of use for biofuel production	Sutherland et al.[14]
a	Proposal of use for anaerobic digestion (other uses: fertilizer, feed)	Sutherland et al.[15]
a	Proposal of use for biofuel production	Sutherland et al.[16]
a	Proposal of use as fertilizer, protein-rich feed or biofuel	Matamoros et al.[17]
a	Proposal of use for fish feeding	Posadas et al.[18]
a	Proposal of use as biofuels and biofertilizers	Posadas et al.[19]
a	Anaerobic digestion of the harvested biomass	Passos et al.[20]
b	Proposal of use for biofertilizers, biofuels and feed	Craggs et al.[21]
b	Not considered	Park and Craggs[22]
c	Not considered	Arbid et al.[23]
c	Not considered	Arbid et al.[24]
d	Anaerobic digestion of the harvested biomass	Bahr et al.[25]
d	Proposal of use as biofuels, biofertilizers, biopolymers, bioplastics, lubricants, paints, dyes and colorants	Ledda et al.[26]
d	Proposal of use as substrate for anaerobic digestion	Alcántara et al.[6]
d	Proposal of use for biodiesel production	Posadas et al.[27]

77 **Figure 1**



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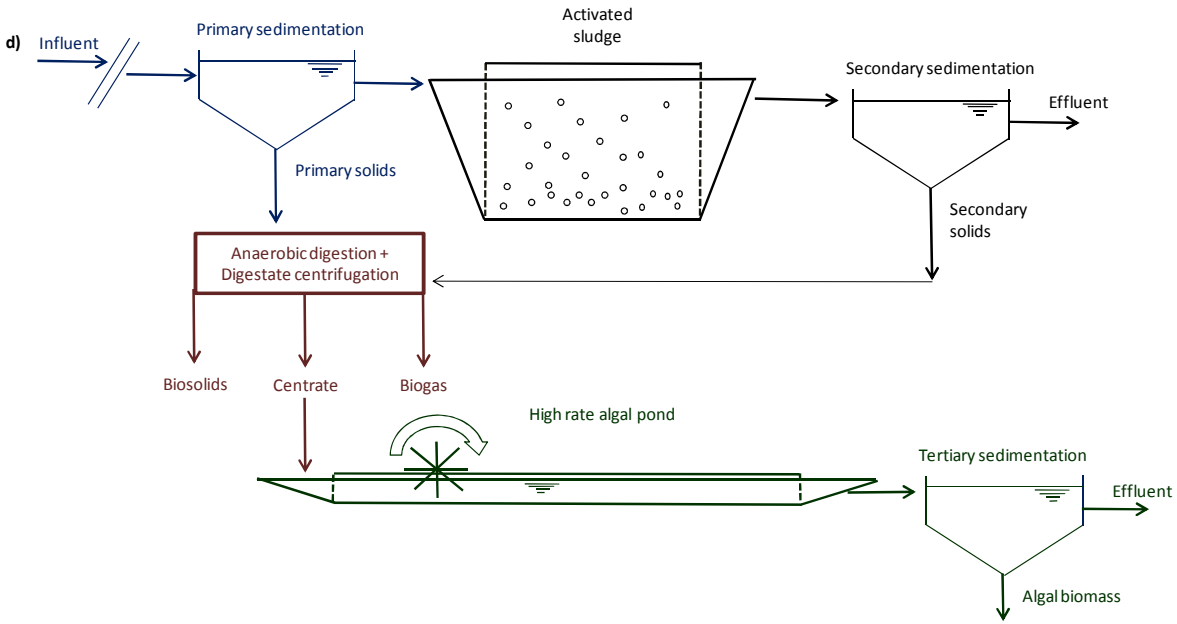


79

80 **Figure 1.** Typical algae-based WWT configurations described in the literature: a) HRAP after primary sedimentation; b) HRAP after advanced

81 facultative pond; c) HRAP after activated sludge processes; d) HRAP for centrate treatment.

MATERIALS AND METHODS

Case study parameters and assumptions

For simplicity, this assessment focuses on algal-based WWT using high rate algal ponds (HRAPs) and all calculations are based on yearly averaged estimates of wastewater characteristics and treatment efficiencies (the conclusions are independent of these simplifications, see below). A domestic wastewater treatment system with a capacity of 2,000 P.E. was used as case study. The characteristics of the raw domestic wastewater are based on the medium strength loads listed by Metcalf and Eddy[28] and the characteristics of the effluent are based on compliance with the EU Directive 2000/60/CE for discharge in sensitive areas[11] (Table 2).

Table 2. Characteristics of the influent wastewater and compliance for effluent discharge according to EU Directive 2000/60/CE[11] for discharge in sensitive areas.

Parameters		Influent	Compliance Directive 2000/60/CE[11]
Average flow rate	**Q (m^3 d^{-1})**	920	NA
Total suspended solids	**TSS (g m^{-3})**	210	35
Chemical oxygen demand	**COD (g m^{-3})**	430	125
Total nitrogen	**TN (g m^{-3})**	40	15
Total phosphorus	**TP (g m^{-3})**	7	2

Selection of algal-based WWT system configurations

Four algal-based WWT system configurations are frequently described in the literature (Fig. 1, Table 1). In these configurations, the algal unit is respectively used for simultaneous carbon and nutrient removal following solid removal during primary treatment (Configuration A, Fig. 1a); nutrient removal following carbon removal during secondary treatment (Configurations B and C, Fig. 1b, and Fig, 1c); and nutrient removal from centrates following the anaerobic digestion (AD) of solids harvested during primary and/or secondary clarification ('centrate' refers to the liquid fraction of

the digestate after centrifugation; Configuration D, Fig. 1d). Configuration 1.a was selected for further assessment because it supports efficient use of energy and land (see Results and Discussion section for full rationale). Two sub-configurations differing in their solid management processes were then compared: The first sub-configuration (Fig 2.a) is one of the most common HRAP integration scheme reported in the literature (Fig. 1a; Table 1) and uses primary settling combined with anaerobic digestion of primary and secondary solids. The nutrient-laden digestate is then transported off-site and irrigated onto land. In the second sub-configuration selected (Fig 2.b), the raw wastewater is pre-treated for grit removal (the solids thus removed are mostly inorganics and landfilled) and the secondary sludge produced during HRAP treatment is dried onsite, stored and used as fertilizer when and where needed.[29] While not the focus of this study (and therefore not part of this assessment), it should be noted that additional treatment units may be needed for compliant wastewater disinfection and/or P removal (Fig. 2). The two sub-configurations selected were designed and operated based on the assumption and criteria showed in Table 3. In both configurations, raw wastewater is elevated in order to gravity-feed the entire treatment system (additional pumping is only required for sludge transfer) and the algal unit is designed as 2 parallel lines of 3 sequential ponds.[21] It was assumed that the existing treatment pond (e.g. typically a 1.5 m deep pond with large HRT e.g. 20 days, 18,400 m^3) will be decommissioned or used for intermittent flow balancing (no costs considered).

Figure 2

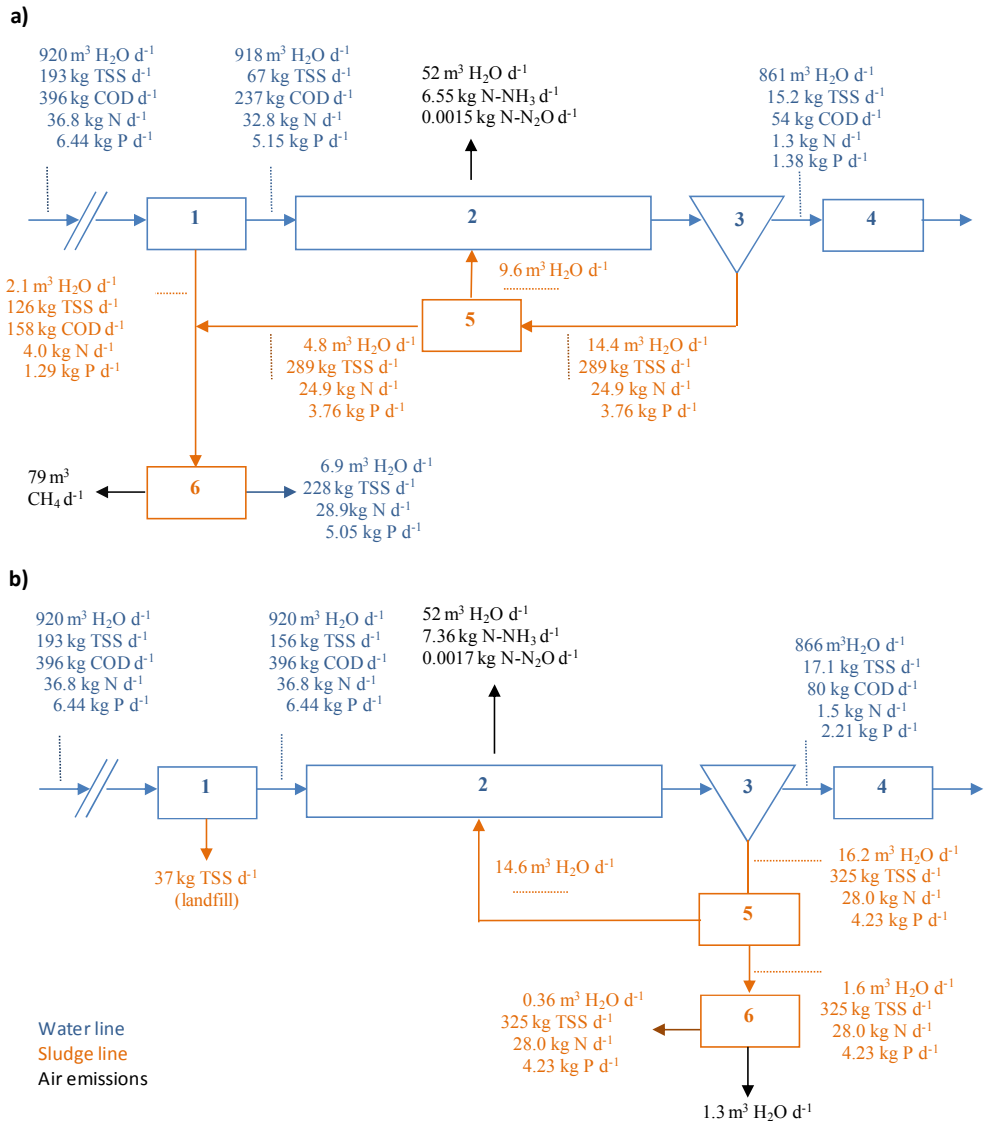


Figure 2. a) Schematic representation and mass balance of HRAP-based WWT system using sludge anaerobic digestion. 1= Primary settler; 2= HRAP; 3= Secondary settler; 4= Disinfection/P removal; 5= Sludge thickening; 6= Anaerobic digestion; b) Schematic representation and mass balance of HRAP-based WWT system using sludge solar drying. 1= Grit removal (TSS are mainly mineral solid), 2=HRAP; 3= Secondary settler; 4=Disinfection and/or P removal; 5= Belt-filter press dewatering; 6= Solar drying.

140 **Table 3**. Operational characteristics sub-configurations 1 and 2 (the performance and
141 design of the HRAP and secondary settler are identical).

	Unit	Characteristics	Reference
Sub-configuration 1 (Fig. 2.a)	Primary sedimentation	Removal efficiencies of 65% TSS, 40% COD, 11% TN and 20% TP, with a final TSS concentration of 6% (w/w) in the primary sludge	Metcalf and Eddy[28]
	Dewatering of algal-bacterial biomass	Sludge thickening from 2% to 6% TSS (w/w)	Metcalf and Eddy[28]
	Anaerobic digestion	Removal of 45% solids and 36% of electricity conversion efficiency	Alzate et al.[34]; Walla and Scheneeberger[35]
Sub-configuration 2 (Fig. 2.b)	Grit removal	Removals of 20% TSS as solid mineral	Metcalf and Eddy[28]
	Dewatering of algal-bacterial biomass	Belt-filter press dewatering from 2% to 20% TSS (w/w)	Metcalf and Eddy[28]
	Solar drying	Solar drying to 90% TSS (w/w) in ventilated greenhouse	Metcalf and Eddy[28]
HRAP	Depth (m)	0.25	Guieysse et al.[31]
	Internal liquid velocity (m s^{-1})	0.2	Guieysse et al.[31]
	HRT (d)	7	Guieysse et al.[31]
	pH	8	Posadas et al.[19]
Secondary clarifier	HRT (h)	10	Park and Craggs[22]
	Depth (m)	4	Craggs et al.[21]
	% TSS Removal efficiency	95	Park et al.[13]

142

143 **Mass balance analysis**

144 In both sub-configurations assessed, the HRAP was sized for complete N removal using
145 a safety factor of approx. 2.0 (see definition in Supporting Information[30]) based on a
146 yearly averaged biomass productivity of 17.7 g total suspended solids (TSS) m^{-2} d^{-1}.
147 This productivity was predicted using a yearly light irradiance (5.44 GJ m^2 y^{-1})
148 representative of a temperate climate,[31] a photosynthetic efficiency of 2.5%,[31] and an
149 algal biomass heat value of 21 KJ $g^{-1.32}$ $N\text{-}NH_4^+$ was considered as the only significant

N source for algal growth and oxygen photosynthetic production was estimated to 1.5 g O_2 g algal biomass photosynthesized^{-1}.[33] Assuming heterotrophic growth can be modelled using a well-mixed steady state model with standard kinetics for aerobic WWT,[28] a yearly average net heterotrophic growth of 0.31 g TSS_{formed} g COD_{used}^{-1} was predicted at the average operational hydraulic retention time (HRT) of 7 d. Heterotrophic productivity was then calculated assuming a total COD removal efficiency of 85%.[19]

While the quantification of greenhouse gas emissions was outside the scope of this study, yearly averaged N-NH_3 and N-N_2O emissions were estimated to 20% and 0.0047% of the influent total nitrogen (TN) loads (Kg d^{-1}), respectively [19, 36], in order to compile accurate N balances (Fig. 2). Heterotrophic and phototrophic dry biomasses were assumed to have a similar N and P content of 8.6% and 1.3% TSS, respectively.[19] The P output loads were calculated based on this composition assuming no other mechanism of P removal than bioassimilation.

The annual water evaporation (0.737 m^3 m^{-2} y^{-1}) under temperate climatic conditions was obtained from Guieysse et al.[31] and the average culture temperature of 13.8ºC was based on the modelling of a pilot-scale HRAP located in Palmerston North (New Zealand) for the year 2013 based local weather data and the model of Béchet et al.[37]. The rate of water losses caused by evaporation and solid management were estimated to 6.4% and 5.8% of the influent flow in configurations 1 and 2 (Fig. 2), respectively, based on Guieysse et al.[31]. The impact of water use was therefore similar for the two configurations considered and not further discussed.

175 **Cost and energy analysis**

176 The average European Union electricity price of 0.208 € KWh^{-1} [38] was used to calculate
177 all electricity expenditures, as well as energy savings from methane production and
178 conversion in sub-configuration 1. As the required pond volumes, areas, and mixing
179 power requirements were nearly identical in the two process configurations considered
180 (Table 4), the costs (capital and operational) and energy demand of the HRAP were
181 estimated based on published literature[21] and only briefly commented (Supporting
182 Information). The capital and operational (maintenance and power) costs of the grit,
183 primary settler, sludge thickener, anaerobic digesters and digestate storage pond
184 (excluding installation costs given their dependence on location), were sourced from the
185 WWTP of Valladolid (Spain), whereas Thermo-System GmbH (Germany) provided
186 estimated prices for the belt-filter press and solar dryer performance (energy
187 consumption for solar drying of 35 kWh ton water $evaporated^{-1}$). The costs for digestate
188 (sub-configuration 1) and biosolids (sub-configuration 2) transport (100 € h of $driving^{-1}$)
189 were based on full scale data collected in Valladolid WWTP (Spain). Manpower costs
190 were estimated to 20 € h^{-1}. The average return distance for digestate and biosolids
191 transportation (50 km which equals 2 hours driving) and the capacity of the truck (8 ton
192 each truck) was provided by Valladolid WWTP. The costs of distributing the
193 digestate/biosolids onto land are not included in this study. Fuel consumption (25 L
194 each 100 km) and costs for truck-based biosolid transport were provided by Valladolid
195 WWTP assuming a fuel energy content of 33.9 MJ L^{-1}.[39] The cost of grit disposal onto
196 landfill was estimated to 120 € ton^{-1}.[40]
197
198 **Sensitivity analysis**

A sensitivity analysis was conducted to study the impact of critical design, operation and economic parameters on treatment costs. As this assessment focuses on the differences between the options considered, parameters that should not affect pond performance specifically to the configuration considered (e.g. photosynthetic efficiency, biomass composition, and meteorological data) were not considered. Emphasis was therefore given to costs associated with solid management and the following parameters were therefore varied: total 'solid management' capital costs (±20% around base values shown in Table 4) to reflect variations in costs due to change in equipment costs and size; biogas to electricity conversion efficiency during anaerobic digestion (from 26% to 42%, base value of 36%[35]); anaerobic digestion efficiency (from 30% to 70%, base value of 45%[41]); sludge thickening efficiency (from 4% to 8%, base value of 6%); belt-filter drying efficiency (from 15% to 25%, base value of 20%); solar drying efficiency (from 85% to 95%, base value of 90%); cost of TSS disposal into the landfill (variation of ±20 € ton^{-1} around base value of 120 € ton^{-1}); transport cost (variation of ±20 € h^{-1} around base value of 100 € h^{-1}); average return distance to land (variation of ±20 km around base value of 50 km); electricity cost (from 0.059 € KWh^{-1} (Kosovo) to 0.304 € KWh^{-1} (Denmark), base value of 0.208 € KWh^{-1} [38]); weekly hours of maintenance (variation of ±20% around based 14 h $week^{-1}$ in configuration 1 and 10 h $week^{-1}$ in configuration 2); and manpower costs (variation of ±20% around base value of 20 € h^{-1}).

RESULTS AND DISCUSSION

Land use and process integration

As shown in Table 4, the land area required for complete N removal was estimated to 12.9 m^2 $P.E.^{-1}$ for both process configurations achieving similar treatment efficiencies

(COD and P removals were slightly better in Configuration 1a due to primary settling, Fig. 2). A safety factor of 2 was used to ensure compliance in winter according to previous experimental work,[19,30] meaning a smaller area is actually needed during summer months. The total amount of oxygen required for complete bCOD oxidation (Table 4) was lower than the amount of oxygen photosynthesized in both configurations. As more land is needed for compliant N (and P) removal via assimilation into algal-bacterial biomass than compliant bCOD removal via partial photosynthetic oxygenation,[19] a HRAP designed for complete N (or P) removal from domestic WW should *de facto* provide free and environmentally-benign bCOD removal. This conclusion explains why configuration a) (Fig. 1a) was selected in the present research: the secondary treatment shown in Figure 1b, 1c and 1d is theoretically redundant if the HRAP is designed for complete N or P removal. This area needed for algal-based secondary treatment is higher than the area required for conventional biological treatment (≈ 0.04 m^2 $P.E.^{-1}$ at 8 h HRT in a 4 m deep activated sludge tank[28]), which limits to use of HRAP in dense urban centres. It is however worse noticing the initial capital investment in land incurred by HRAPs would remain, or even increase, in the long term,[33] and this specific costs was therefore not included in our assessment. Given the large excess in oxygenation capacity generated by algae photosynthesis, the removal of biodegradable suspended solids (bSS) via primary settling (sub-configuration 1) is unlikely necessary. As shown in Figure 2, primary settling also contributed to little TN removal (because most of the influent TN is found as NH_4^+) and, therefore, had little impact on HRAP design. The higher bCOD load thus applied to the HRAP in sub-configuration 2 (Fig. 2.b) is predicted to support a higher heterotrophic biomass productivity than in HRAP 1 (4.8 g m^{-2} d^{-1} compared to 2.9 g m^{-2} d^{-1}). High density suspended solids should be still removed from the raw wastewater

prior to HRAP treatment as it would be impractical to frequently remove sediments from large lined and lightly-mixed ponds.

Table 4. Main features and projected wastewater treatment costs in two algal-based WWT sub-configurations.

		Configuration	
		1	**2**
HRAP Design[1]	Illuminated area (m^2)	25701	25760
	Working volume (m^3)	6425	6440
	O_2 required for COD oxidation (kg O_2 d^{-1})	113	188
	O_2 produced by photosynthesis (kg O_2 d^{-1})	347	330
HRAP Costs	Construction (€)[2]	219,276	219,775
	Energy for mixing and pumping (€ y^{-1})	6,511	6,511
	Total HRAP over life (€)[2, 3]	298,433	298,932
Energy expenditure	Pumping (kWh m^{-3} WW)	0.015	0.015
	HRAP mixing (kWh m^{-3} WW)	0.039	0.039
	Biosolids transport (kWh m^{-3} WW)	0.114	0.006
	Biogas production (kWh m^{-3} WW)	0.307	0
Costs solid management	Equipment (€)	435,000[4]	450,000[5]
	Maintenance (€ y^{-1})	14,600	10,400
	Electricity (€ y^{-1})	7,300	3,344
	Digestate (1) or biosolids (2) transport (€ y^{-1})[6]	65,000	3,400
	TSS disposal (grit removal) (€ y^{-1})[7]	0	1,622
	Energy savings (€ y^{-1})	- 21,442	0
	Total operating-biosolids costs (€ y^{-1})	65,457	18,765
	Total solid management costs over life (€)[3]	1,529,274	977,089
Total costs	Total operating costs (€ y^{-1})	71,968	25,276
	Total costs (€/m^3 WW)[3]	0.228	0.145

[1]: In both configurations, the algal unit was designed as 2 series of 3 ponds with individual channel length and width of 358 and 6 m, respectively.
[2]: HRAP + secondary settler, the capital costs of HRAP construction were estimated to 8.5 € m^{-2} (land purchase is excluded). [21]
[3]: Net Present Value assuming a discount rate of 6% and a project life of 20 years; extra costs for equipment installation and monitoring are not included.
[4]: Primary settler (150,000 €), sludge thickener (50,000€), anaerobic digesters (200,000 €), and digestate storage pond (35,000 €).
[5]: Grit removal (150,000 €), belt-filter press (50,000 €) and solar dryer (250,000 €).
[6]: Costs for disposal onto land are not included.
[7]: Cost for transportation and disposal onto landfill.

Energy use

In the process configurations assessed, the energy required for elevating raw wastewater was estimated to 0.015 kWh m^{-3} WW treated, while the energy demand for pond mixing was estimated to 0.039 kWh m^{-3} WW treated, or 0.018 kWh $P.E.^{-1}$ d^{-1} (Table 4, Supporting Information), which compares favourably against the energy demand for mechanical mixing and aeration during conventional secondary treatment (0.15 – 0.62 kWh m^{-3} WW treated[42]). Despite energy production via anaerobic digestion (0.307 kWh m^{-3} WW treated), the energy consumption for digestate transport in sub-configuration 1 was 19 times higher than the energy required for the transport of dried biosolids in sub-configuration 2 (Table 4).

Biosolid management

Sub-configuration 1

The anaerobic digestion of 126 kg TSS d^{-1} of primary sludge and 289 kg TSS d^{-1} of algal-bacterial biomass was predicted to generate 79 m^3 of CH_4 daily (Fig. 2.a), or 282 KWh d^{-1} as electricity. This equates to potential energy savings of 21,442 € per year, or 10.7 € yearly per P.E., which is lower than the maintenance and electricity cost for AD operation (11 € $P.E.^{-1}$ y^{-1}), not including the cost of biogas storage and purification (raw biogas typically contains 40-75% CH_4, 25-60% CO_2, and various impurities such as 0.005-2% H_2S). According to most current European Directives for biogas use, biogas injected into the natural gas grid should contain > 95% CH_4 and less than 2% CO_2 and 0.5%, O_2.[43] Given the low flowrate predicted in our case study (average of 3.3 m^3 raw biogas h^{-1}), biogas would be likely burnt using a microturbine meaning purification may

not be necessary.[44] The cost of the microturbine was not included as it was predicted to be negligible compared to the rest of the equipment (Table 4).

Anaerobic digestion was also predicted to generate 6.9 m^3 digestate d^{-1} containing 228 kg TSS (3.3% content of biosolids (w/w)), 28.9 kg N and 5.1 kg P (Fig. 2.a). The land application of 2,599 m^3 digestate y^{-1} can require considerable storage when soils are saturated and/or plant nutrient uptake is minimum, and considerable areas of suitable land (e. g. 52 ha at a typical N land loading of 200 kg N ha y^{-1} [45]) near the WWT facility to reduce transport costs and impacts. A 6-month capacity pond of 1300 m^3 was therefore budgeted to enable digestate storage when land application within a 50 km radius is not feasible (in practice, such pond may need to be periodically aerated for odour control, but this potential additional cost was not included). At an average return distance for digestate disposal of 50 Km, the total cost of digestate transport was projected to approximately 65,000 € per year (32.5 € $P.E^{-1}$ y^{-1}). While biosolid disposal onto landfill may be feasible, this may require further dewatering in sub-configuration 1 to reduce transport costs and landfill fees; this alternative was therefore not considered.

Sub-configuration 2

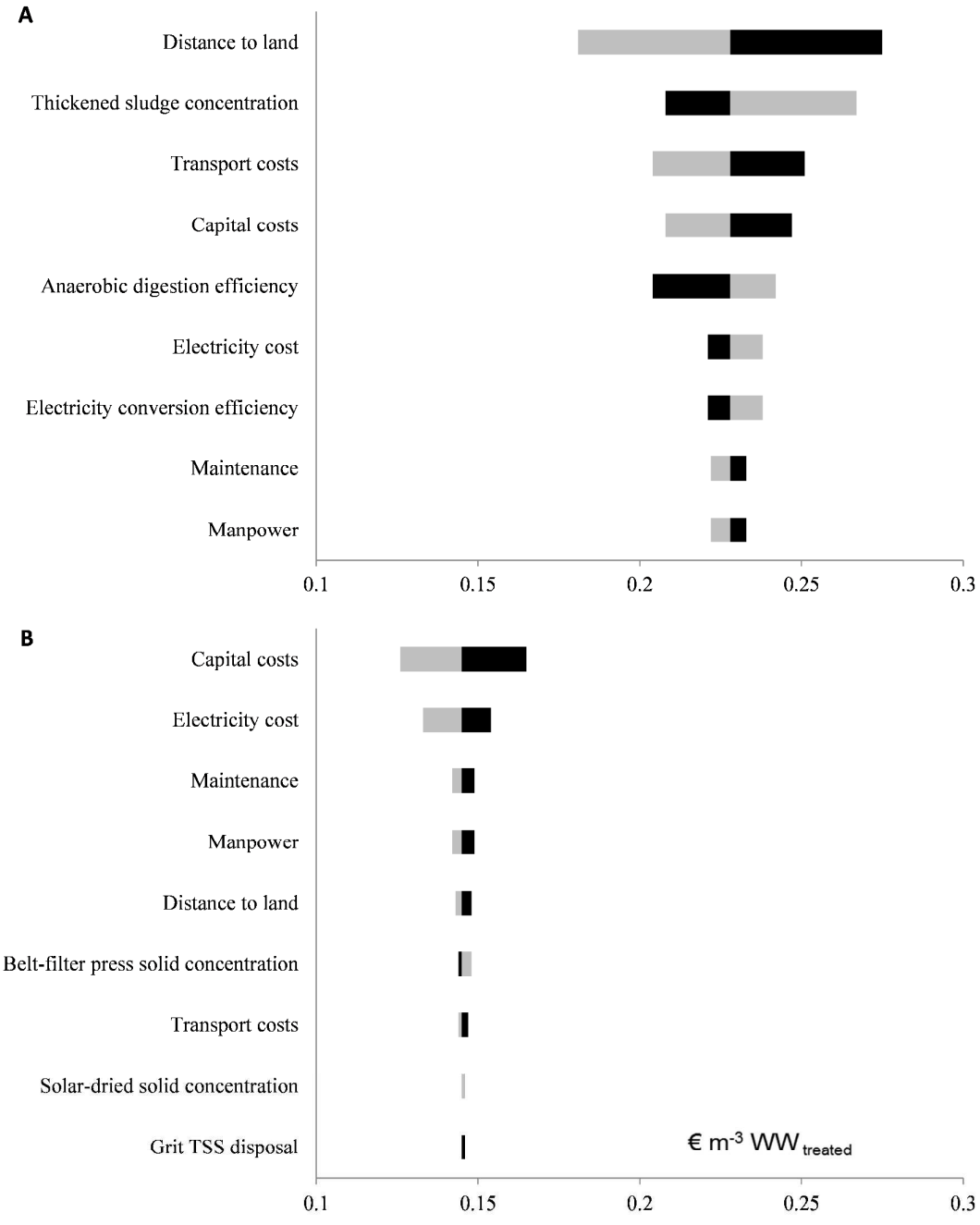
Solid management in sub-configuration 2 (Fig. 2.b) is based the rapid pre-drying of the algal-bacterial sludge using a belt-filter press (with recirculation of the removed wastewater to the HRAP) to minimize nutrient release into the aqueous phase (unlike during anaerobic digestion where a significant amount of nutrient is dissolved) followed by solar drying. The yearly dried sludge production was thus estimated to 132 ton. This sludge can be safely stored and used as soil fertilizer when and where necessary.[29] Operational costs for manpower and electricity were estimated to 10,400 € and 3,340 €,

313 respectively (Table 4). Thus, belt-filter press and solar drying operation cost 6.9 € $P.E.^{-1}$
314 y^{-1}. At a return distance of 50 Km, solid transport and disposal would cost of 3,400 € y^{-1}
315 (1.7 € $P.E.^{-1}$ y^{-1}). The disposal costs of TSS removed in the grit tank (13.5 ton y^{-1}) were
316 estimated to 1,622 € y^{-1} (0.8 € $P.E.^{-1}$ y^{-1}).
317
318 The total costs (capital + operational) for biosolids management, were thus estimated to
319 1,529,274 € (38.2 € $P.E^{-1}$ y^{-1}) and 977,089 € (24.4 € $P.E^{-1}$ y^{-1}) for sub-configurations 1
320 and 2, respectively (Table 4). Sub-configuration 2 is therefore more economical than
321 sub-configuration 1 and this is largely due to a drastic reduction in biosolids transport
322 costs. The total operational costs (including HRAP operation) were estimated to 0.228 €
323 m^{-3} WW treated and 0.145 € m^{-3} WW treated for sub-configurations 1 and 2,
324 respectively (Table 4).
325
326 **Sensitivity analysis**
327 As explained above, the HRAP design and operation were nearly identical in the two
328 process configurations assessed. The sensitivity of parameters solely associated to this
329 operational unit on the results was therefore not estimated and emphasis was given to
330 solid management costs.
331
332 As illustrated in figure 3a, the costs of sub-configuration 1 (anaerobic digestion) were
333 highly sensitive to costs related to digestate transport (e.g. direct transport costs but also
334 indirect costs such as dewatering and digestion efficiencies) and were predicted to
335 remain higher than the costs of sub-configuration 2 (solar drying) even under favourable
336 economic (high electricity costs) or technical (high anaerobic digestion efficiency)
337 conditions and when the capital costs of the later are increased by 20%. In comparison,

the costs of sub-configuration 2 were rather insensitive to all parameters considered expected for capital costs, and this is largely explained by the high energy efficiency of solar drying generating low volumes of waste for off-site disposal.

It can therefore be safely concluded that the anaerobic digestion of algal-bacterial biomass generated during wastewater is neither economically profitable for the sole purpose of generating power (due to the low value of the energy produced against the high additional costs incurred by digestate management) nor competitive against alternative algal-based wastewater treatment processes with more efficient solid management strategies. An exception could be made if sufficient digesting capacity is already available but this situation may seldom arise because algal-bacterial secondary treatment generates far more biosolids than conventional treatment using activated sludge (e.g. 415 Kg TSS d^{-1} against 218 Kg TSS d^{-1} in our simulation, primary sludge included). The good news is, however, that algal-based wastewater treatment combined with rapid solar drying appears as a cost-effective option to achieve nutrient removal in rural areas.

363 **Figure 3**



364

365 **Figure 3.** Sensitivity analysis of WWT cost (€ m^{-3} WW treated) respect to the base case
366 in **A**) sub-configuration 1 and **B**) sub-configuration 2. For each parameter, the dark bar
367 represents the upper value of the range used in the simulation, while the light bar
368 corresponds to the lower value of the range. All costs were estimated assuming a

discount rate of 6% and a project life of 20 years (extra costs for equipment installation and monitoring are not included).

ACKNOWLEGMENTS

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514 GRAPHICAL ABSTRACT

515

SUPPORTING INFORMATION

Nutrient removal and solid management restrict the feasibility of algal biofuel generation via wastewater treatment

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CONTENT

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Table S1. Typical algae-based domestic WWT configurations described in published laboratory experiments considering biosolid management (ultimate biosolid disposal was however seldom discussed).

Process configuration (Figure 1)	Biosolids management	Reference
a	Anaerobic digestion	Ometto et al.[S1]
a	Anaerobic digestion	González Fernández et al.[S2]
a	Biofuel production proposed but not demonstrated (fatty acid analysis)	Jebali et al.[S3]
a	Dark fermentation for biohydrogen production	Batista et al.[S4]
a	Anaerobic digestion	Gutiérrez et al.[S5]
a	Anaerobic digestion	Kinnunen et al.[S6]
a, d	Anaerobic digestion or transterification for biodiesel production	Caporgno et al.[S7]
a, d	Proposal of different uses depending on the biomass composition	Cho et al.[S8]
d	Various proposals depending on the biomass composition	Morales-Amaral et al.[S9]
d	Various proposal depending on the biomass composition	Sepúlveda et al. [S10]

Table S2. Typical algae-based domestic WWT configurations described in published theoretical studies and reviews considering biosolid management (ultimate biosolid disposal was however seldom discussed).

Process configuration (Figure 1)	Biosolids management	Reference
Integration of aquaculture in all the possible scenarios	Anaerobic digestion, biofertilizer, and biosorbent	Steele et al.[S11]
General application of aquaculture in WWT	Biofuels production	Abinandan and Shanthakumar [S12]
General application of aquaculture in WWT	Biofuels production	Judd et al. [S13]
a	Biofuels production	Acién et al.[S14]
a	Biofuels production	Razzak et al.[S15]
c	Biofuels production or direct combustion	Sturm and Lamer[S16]
c	Biogas, biodiesel and/or biofertilizer production	Drexler et al.[S17]
Modification of process d*	Anaerobic digestion	Menger-Krug et al.[S18]

*(Additional nutrients are supplied by primary treated wastewater)

SAFETY FACTOR DEFINITON

According to Rittman and McCarty[S19], the safety factor for aerobic heterotrophic bCOD removal (SF_{het}) can be defined as the ratio of the operational solids retention time (SRT) (e.g. 7 days in our case study as SRT = hydraulic retention time (HRT) in well mixed systems without cell recycled or attachment) to the minimum SRT (SRT_{min}, d^{-1}) value needed to prevent the washout of aerobic heterotrophs. The SRT_{min} is therefore computed as:

$$\frac{1}{SRT_{min}} = \mu_{max} \frac{S_0 K_s}{K_s + S_0} - k_d \quad \text{(S1)}$$

Where S_0 (219.7 g bCOD m^{-3}) is the concentration of bCOD in the pond influent, μ_{max} (3.95 d^{-1} at 13.8˚C) is the maximum specific growth rate of aerobic heterotrophs, K_S (20 g bCOD m^{-3}) is the substrate saturation constant of aerobic heterotrophs and k_d (0.094 d^{-1} at 13.8˚C) is the rate of microbial decay of aerobic heterotrophs. In our simulation, SF_{het} was thus determined to 25 (using typical kinetic parameters and temperature correction factors for domestic wastewater treatment), which is within the range of safety factors used for conventional secondary wastewater treatment[S19].

If the case of ponds relying on photosynthetic aeration, a safety factor for phototrophs (SF_{ph}) based on the maximum photosynthetic growth rate can be defined as (Rittman and MacCarty[S19]):

$$SF_{ph} = \mu_{m,ph} \cdot HRT \quad \text{(S2)}$$

Where $\mu_{m,ph}$ is the maximum phototroph growth rate (d^{-1}) estimated as:

$$\mu_{m,ph} = K_p \cdot T \quad \text{(S3)}$$

Where K_p equals 0.10 ± 0.025 d^{-1}˚C^{-1} and T = 13.8˚C in our case study. SF_{ph} was thus estimated to 9.66, which is within the range of 3-10 cited by Rittman and MacCarty[S19] and confirms the large excess of photosynthetic oxygenation capacity of the design.

Following the same approach, a SF for nitrogen removal via assimilation can be defined as the ratio of the design pond area versus the minimum pond area required for complete N assimilation. Based on yearly averages, this safety factor was set to 2 to ensure compliance during periods of low productivity. A more in depth assessment of this parameter was beyond the scope of our study.

ENERGY CALCULATIONS

The hydraulic power (P, KW) of the pump was calculated as:

$$P = q \cdot \rho \cdot h \cdot g/(3.6 \cdot 10^6) \cdot E \quad \text{(S4)}$$

Where q is the flow capacity ($m^3\ h^{-1}$), ρ the density of the fluid (≈ 1005 kg m^{-3}), h the differential head (estimated approx. 4 m), g is the acceleration of gravity (9.81 m s^{-2}) and E the efficiency of the pump (75%).[S20]

The amount of energy required for mixing the HRAP was calculated according to Borowitzka[S21] as:

$$P = \frac{Q \cdot W \cdot \Delta d}{102 \cdot e} \quad \text{(S5)}$$

Where Q is the quantity of water in motion ($m^3\ s^{-1}$), W the density of water (kg m^{-3}), e the efficiency of the paddle wheel (0.17) and 102 is the conversion factor to convert $m \cdot kg \cdot s^{-1}$ to KW. Finally, a Manning coefficient of 0.012 (smooth plastic on granular earth) was considered. [S21] In both configurations, the algal unit was designed as 2 series of 3 ponds with individual channel length and width of 358 and 6 m, respectively.

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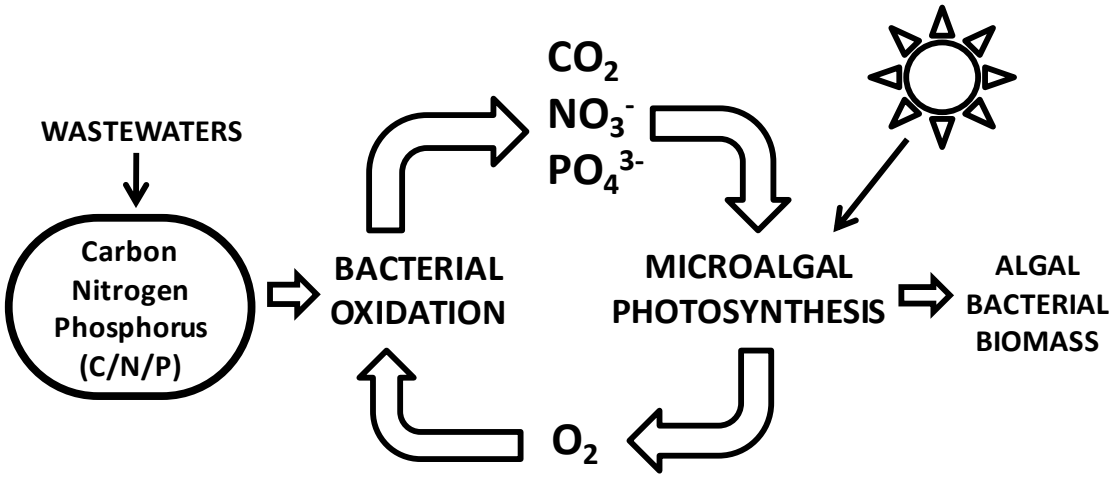
Conclusions and future work

Chapter 10



The results obtained in the present thesis confirmed the potential of algal-bacterial processes for WWT and the possibility of implementing this biotechnology in an efficient, economic and environmentally friendly way. Thus, microalgae-based processes are gradually being accepted as a real alternative to conventional WWT technologies.

The high NH_4^+ concentrations in some agroindustrial WWs or/and the high pH reached during their treatment in algal-bacterial processes inhibited microbial activity, which limited WWT performance. In this context, some WWs should be diluted prior to treatment in algal-bacterial photobioreactors or the pH should be controlled (**Chapter 3**). Likewise, the optimum C/N/P ratio for WW treatment was estimated to 100/18/2 (g/g/g), biodegradable carbon being the main limiting component in the agroindustrial WW evaluated (**Chapters 3-8**).

The scratching of the surface to harvest the biomass in open biofilm photobioreactors constituted an easy task. The performance of algal-bacterial biofilm photobioreactors at full scale should be tested to carry out an economic analysis, despite their successful performance in terms of biomass harvesting at laboratory conditions (**Chapters 4-5**). Likewise, biomass harvesting by natural gravity sedimentation in conical settlers at HRTs lower than 10 h was also proven as a suitable and economic alternative to recover the biomass in HRAPs and to comply with the regulations for WW disposal in terms of TSS (**Chapters 6-7**). These successful results rendered gravity sedimentation as an economic alternative compared to conventional physical/chemical technologies, which is expected to reduce the current high cost of biomass harvesting.

Chapters 4 and 5 revealed that the high removal of C and N by stripping and the high water footprint in open algal-bacterial biofilm photobioreactors can eventually jeopardize their implementation at full scale. Similarly, the results presented in Chapter 5 showed the necessity to improve the design of enclosed biofilm photobioreactors with diameters of the tubes higher than 1 cm to avoid a rapid biomass clogging. Stripping and assimilation into algal-bacterial biomass were also the main mechanisms underlying C and N removal in HRAPs, while phosphorus at the optimal pH of HRAP operation (7-9) was only removed by assimilation into biomass (**Chapters 6-8**). Nitrifying bacteria contributed to decrease NH_3 volatilization, which led to a NO_3^- mediated total nitrogen sequestration in the growth medium and to lower TN removal efficiencies in WWs with low C/N ratio (**Chapters 4, 5, 7 and 8**). The high water footprint of our pilot HRAPs induced by the high turbulence prevailing in the cultivation broth deteriorated the quality of the final treated effluent (**Chapters 6 and 8**). However, and despite this disadvantage, HRAPs currently constitutes the most realistic photobioreactor

configuration for the implementation of microalgae-based processes in conventional WWTPs (**Chapters 6-9**).

Microalgae population in photobioreactors was very diverse regardless of the operational conditions (**Chapters 5 and 7**). *Phormidium*, *Scenedesmus* and *Chlorella* were identified during centrate and primary domestic WWT in open and enclosed algal-bacterial biofilm photobioreactors (**Chapter 5**) and in HRAPs devoted to WWT (centrate or vinasse) coupled with biogas upgrading (**Chapter 7**). Therefore, these microalgae represent the best candidates for future inoculations of WWT photobioreactors. Likewise, a higher microalgae diversity was found in the open algal-bacterial biofilm photobioreactor than in its enclosed counterpart as a result of its higher risk of contamination (**Chapter 5**). Similarly, a high bacterial diversity was also recorded during vinasse treatment and biogas upgrading in a 180 L HRAP, although no correlation between microalgae and bacteria species was identified (**Chapter 7.2**). These results represented a step-forward in the understanding of microalgae-bacteria population dynamics in algal-bacterial photobioreactors.

Biogas upgrading in HRAPs coupled with WWT was proven as an effective alternative compared to commercial biogas upgrading technologies. A HRAP interconnected to an external absorption column via cultivation broth recirculation was successfully evaluated to carry out this simultaneous treatment. The operational optimization of this experimental set-up allowed the removal of 99% of the CO_2 contained in the biogas during the treatment of diluted centrate (**Chapter 7.1**). On the other hand, the O_2 concentration in the purified biogas decreased from 20% to 0.7±0.2% when raw vinasse without dilution was fed at the bottom of the absorption column (**Chapter 7.2**). However, further improvements are still necessary to decrease the N_2 desorbed from the recirculation algal broth into the upgraded biogas and to achieve the required CH_4 purity for injection into natural gas grids or use as automotive fuel (≥95%) (**Chapter 7.2**).

Similarly, the simultaneous WW and flue gas treatment by microalgae-based processes was shown as an economic alternative compared to conventional CO_2 mitigating technologies. This process was implemented in HRAPs constructed with open sumps, where the flue gas was bubbled. However, this experimental set-up resulted in low CO_2 mass transfer and high removal rate of carbon by stripping. Thus, most of the CO_2 transferred from the gas to the liquid phase was then removed by stripping, which did not result in a higher biomass productivity and, consequently, in a higher nutrient removal efficiency, despite pH control was effective (**Chapter 8**). In this context, the influence of pH (in the range from 7 to 9) on WWT,

biomass productivity and biomass composition in the outdoors HRAPs was negligible (**Chapter 8**). Likewise, a slightly superior WWT performance was recorded when flue gas instead of pure CO_2 was used for pH control. Furthermore, the use of flue gas instead of pure CO_2 for pH control resulted in a higher lipid and C, N and P content in the harvested biomass (**Chapter 8**).

The C, N and P content of the algal-bacterial biomass was slightly modified depending on the nature of the WW and its initial C/N/P ratio (**Chapters 3-8**). However, despite the above mentioned variations, the C, N and P content of the algal-bacterial biomass was similar regardless of the operational conditions (WW type, photobioreactor configuration, HRT, etc), with values ranging from 40% to 60%, from 5% to 9% and from 0.5% to 2%, respectively (**Chapters 3-8**). On the other hand, process operation under nutrient starvation did not increase the biomass lipid content, which remained at 2.9-11.2 % (**Chapter 7.1**). As previously discussed, CO_2 addition from flue gas significantly influenced the biochemical composition of the harvested biomass (**Chapter 8**).

Finally, the case study evaluated in **Chapter 9** showed that algal-bacterial processes should be preferentially used as secondary treatment for combined C and nutrient removal, when integrating HRAPs in a conventional WWTP. In this context, primary settling was unlikely necessary based on the large excess in oxygenation capacity generated by algae photosynthesis for complete organic matter oxidation. Therefore, a grit for fixed suspended solids removal was considered as an optimum alternative prior to HRAP. Likewise, the evaluation of the alternatives for an efficient biosolids treatment and management showed that a solar drier after algal-bacterial biomass secondary settling and dewatering in a belt press (to use biomass as a biofertilizer) was a more sustainable alternative than anaerobic digestion of the primary sludge and algal-bacterial biomass.

Despite the advances carried out in the present thesis for the application of algal-bacterial processes at full scale in WWTPs, a further optimization of the operational conditions and a deeper knowledge of the interactions between microalgae and bacteria should be achieved. Based on the results here obtained, future research in the field of microalgae-based WWT should focus on:

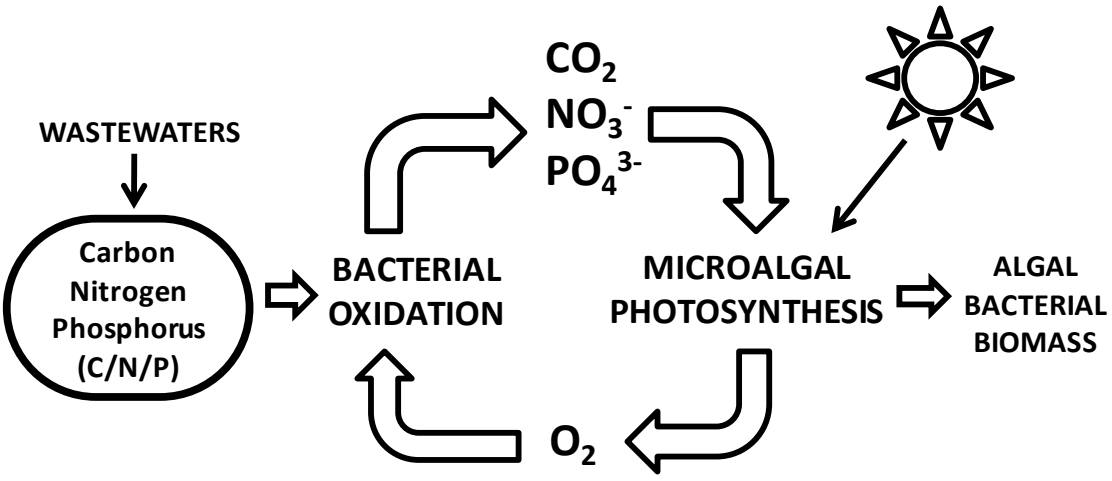
- The optimization in the design of open algal-bacterial biofilm photobioreactors to decrease the high C and N removal by stripping and their high water footprint. This will further increase biomass productivity and improve the quality of the final treated effluent. In this context, novel designs of enclosed tubular algal-bacterial biofilm

photobioreactors can also contribute to overcome the main limitations of open algal-bacterial biofilm photobioreactors here identified.

- The scale-up of biofilm photobioreactors, which will provide a more realistic estimation of their harvesting costs compared to those of conventional HRAPs.
- Evaluation of alternatives to decrease the water evaporation in HRAPs in order to avoid the deterioration of the quality of the treated effluent.
- The selection of efficient microalgae and bacteria consortia capable of supporting a consistent WWT, which would lead to robust inocula and reduced process start-up periods.
- The optimization of the integration of WWT in HRAPs and biogas upgrading (mainly to increase CH_4 purity) in an absorption unit, which will represent an important breakthrough in terms of bioenergy recovery. In this context, the performance of this experimental set-up under outdoors conditions and in enclosed photobioreactors (the latter to avoid N_2 desorption) should be tested.
- Development of different designs of sumps in HRAPs in order to improve the efficiency of CO_2 mass transfer from flue gas to the algal cultivation.
- Minimization of NH_3 volatilization in WWT photobioreactors. Despite the low emissions of N_2O in HRAPs, the indirect GHG emissions mediated by NH_3 stripping (conversion factor of 0.01 Kg N-N_2O Kg N-NH_3^{-1}) can compromise the environmental sustainability of algal-bacterial processes.
- A comparative life cycle analysis of conventional WWT HRAPs, the novel algal-bacterial photobioreactor configurations here evaluated and conventional technologies for WWT in order to provide new insights on the economic and environmental sustainability of microalgae-based WWT technology.

About the author

Chapter 11



Biography

Esther Posadas Olmos (Segovia, 1987) started her Chemical Engineering studies in 2005 at the University of Valladolid. Between 2010 and 2011, Esther spent 7 months at Tampere University of Applied Sciences (TAMK) (Finland) within the frame of the Erasmus Program successfully developing her Final Year Project on *Preservation of biowaste for reuse* (PREBIORE). Esther Posadas graduated in March 2011 and joined the Gas treatment-Microalgae Research Group headed by Dr. Raúl Muñoz in the Environmental Technology Research Group (Department of Chemical Engineering and Environmental Technology- University of Valladolid). During two years she carried out research in a project funded by the company Biogas Fuel Cell S.A, and in April 2013 she was awarded with a PhD Grant by the University of Valladolid - Banco Santander.

Her PhD thesis initially focused on the study of the basic interactions between microalgae and bacteria during the batch treatment of different agroindustrial wastewaters. After this initial proof of concept, the experimental work of Esther centered around the continuous operation of multiple photobioreactor configurations treating several types of wastewaters in order to determine the main potential, limitation and challenges of algal-bacterial processes in wastewater management. The candidate carried out within her PhD studies one six-months research stay (July – December, 2013) at University of Almería (Spain). Esther operated semi-industrial raceways devoted to secondary domestic wastewater treatment under the supervision of Dr. Emilio Molina and Dr. Francisco Gabriel Acién. Finally, she also carried out a four-months experimental research stay (August – December, 2015) at Massey University (Palmerston North, New Zealand) focused on the integration of high rate algal ponds in conventional wastewater treatment plants under the supervision of Dr. Benoit Guieysse.

Publication list

1. **Posadas E.**, Plouviez M., Muñoz R., Guieysse B. (2016). Nutrient removal and solid management restrict the feasibility of algal biofuels generation via wastewater treatment. Submitted for publication to Environmental Science and Technology.
2. **Posadas E.**, Szpak D., Lombó F., Domínguez A., Díaz I., Blanco S., García-Encina P.A., Muñoz R. (2016). Feasibility study of biogas upgrading coupled with nutrient removal from anaerobic effluents using microalgae-based processes, J. Appl. Phycol. DOI: 10.1007/s10811-015-0758-3.
3. **Posadas E.**, Serejo M. L., Blanco S., Pérez R., García Encina P.A., Muñoz R (2015). Minimization of Biomethane Oxygen Concentration during Biogas Upgrading in Algal-Bacterial Photobioreactors, Algal. Res 12: 221-229.
4. **Posadas E.**, Muñoz A., García-González M.C., Muñoz R., García-Encina P.A. (2015). A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters. J. Chem. Technol. Biotechnol. 90: 1094-1101.
5. Serejo M.L., **Posadas E**., Boncz M.A., Blanco S., García Encina P.A., Muñoz R. (2015). Influence of biogas flow rate on biomass composition during the optimization of biogas upgrading in microalgal-bacterial processes, Env. Sci. Technol. 49: 3228-3236.
6. **Posadas E.**, Morales M.M., Gómez C., Acién F. G., Muñoz R. (2015). Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways, Chem. Eng. J. 265: 239-248.
7. **Posadas E.**, García-Encina P.A, Dominguez A., Diaz I., Becares E., Blanco S., Muñoz R. (2014). Enclosed tubular and open algal-bacterial biofilm photobioreactors for carbon and nutrient removal from domestic wastewater. Ecol. Eng.67: 156-164.
8. **Posadas E.**, Bochon S., Coca M., González M.C., García-Encina P.A, Muñoz R. (2014). Microalgae-based agroindustrial wastewater treatment: a preliminary screening of biodegradability. J. Appl. Phycol. 26: 2335-2345.
9. **Posadas E.**, García-Encina P.A, Soltau A., Domínguez A., Díaz I., Muñoz R. (2013). Carbon and nutrient removal from centrates and domestic wastewater using algal-bacterial biofilm bioreactors. Bioresour. Technol. 139: 50-58.

Book Chapters

1. Alcántara C., **Posadas E.**, Guieysse B., Muñoz R. (2015). Microalgae-based waste water treatment. In: Handbook of Microalgae: Biotechnology Advances. Edited by Se-Kwon K, Elsevier. Chapter 29; pp. 439-452.

2. **Posadas E.**, Alcántara C., García-Encina P., Guieysse B., Norvill Z., Gouveia L., Congestri R., Acién Fernández F. G., Markou G, Koreivienė J., Muñoz R. (2017). Microalgae cultivation in wastewater. In: Microalgae based biofuels and bioproducts. Elsevier (In preparation).

Conferences

1. **Posadas E.**, Serejo M. L., Blanco S., Pérez R., García Encina P. A., Muñoz R., Biogas upgrading and vinasse treatment by microalgae-based processes: minimizing O_2 concentration, World Congress on Anaerobic Digestion, 15-18 November 2015, Viña del Mar (Chile) (Oral Presentation).
2. **Posadas E**., Carbon and nutrient removal during algal wastewater treatment, The NZ Symposium on Algae and Cyanobacteria, Cawthron 24 August 2015 (Nelson, New Zealand) (Oral Presentation).
3. **Posadas E.**, Influence of temperature and light on algal-bacterial wastewater treatment systems, Oral presentation in the workshop Implementation of microalgae in waste water treatment, current challenges: from research to application; IWA 2015, 7-10 June 2015, Vasteras (Sweden).
4. Serejo M. L., **Posadas E.**, Boncz M.A., García-Encina P.A., Muñoz R., Influence of liquid recirculation on CO_2 and H_2S removal efficiency and O_2 biomethane content during biogas upgrading by microalgal-bacterial process, IWA 2015, 7-10 June 2015, Vasteras (Sweden) (Oral Presentation).
5. Serejo M.L., **Posadas E.**, Boncz M.A., Blanco S., García.-Encina P. A., Muñoz R., Impact on biomass composition during the optimization of the integral biogas upgrading in microalgal-bacterial processes, IWA 2015, 7-10 June 2015, Vasteras (Sweden) (Oral Presentation).
6. **Posadas E.**, Morales M.M., Gómez C., Acién F.G., Muñoz R., Secondary domestic wastewater treatment in outdoors pilot raceways by algal-bacterial processes: pH and CO_2 source influence, IWA 2015, 7-10 June 2015, Vasteras (Sweden) (Oral Presentation).
7. **Posadas E.**, Serejo M.L., García-Encina P.A., Muñoz R., Microalgae-based processes applied to simultaneous biogas upgrading, wastewater treatment and biomass revalorization. EMBS, 2014, Euromediterranean Microalgal Biotechnology Seminar & Worskshop. 20-24 October 2014, Almería (Spain) (Oral Presentation).
8. Morales M.M., **Posadas E.**, Gómez C., Acién F.G., Fernández-Sevilla J.M., Muñoz R., Molina E., Limiting factors in wastewater treatment using Microalgae-bacteria consortium. 5th Congress of the International Society for Applied Phycology. 22-27 June 2014. Sydney, Australia (Oral Presentation).

9. **Posadas E.**, Szpak D., Lombó F., Domínguez A., Díaz I., Blanco S., García-Encina P.A., Muñoz R., Biogas upgrading coupled with nutrient removal from centrates and lipid accumulation using microalgae-based processes. 10th International Conference on Renewable Resources and Biorefineries. 4-6 June 2014. Vallladolid 2014 (Spain) (Oral Presentation).

10. **Posadas E.**, García-Encina P.A., García-Gonzalez M.C., Muñoz R., Algal-bacterial biomass removal from fish farm wastewater treatment using coagulation/flocculation processes. 10th International Conference on Renewable Resources and Biorefineries. 4-6 June 2014. Vallladolid 2014 (Spain) (Poster).

11. **Posadas E.**, García-Encina P.A., Domínguez A., Díaz I., Becares E., Blanco S., Muñoz R., Microalgae-based domestic wastewater treatment in enclosed tubular and open biofilm photobioreactors. YAS 2014: Young Algaeneers Symposium 2014. 3-5 Apr 2014, Montpellier-Narbonne (France) (Oral Presentation).

12. **Posadas E.**, García-González M.C., Muñoz R., García-Encina P.A., Diurnal variations in a High Rate Algal Pond treating fish farm wastewater. YAS 2014: Young Algaeneers Symposium 2014. 3-5 Apr 2014, Montpellier-Narbonne (France) (Poster).

13. **Posadas E.**, Morales M.M., Gómez C., Muñoz R., Acién F.G., Microalgae-based processes applied to the simultaneous treatment of domestic wastewater and CO_2 removal from flue gas. II Mini-Simposio de Investigación en Ciencias Experimentales, 15 November 2013, Almería (Spain) (Poster).

14. **Posadas E,** Soltau A., García-Encina P. A., Domínguez Díaz I., Muñoz R., Biofilm bioreactors for carbon and nutrient removal from centrates and domestic wastewater. III Congreso Latinoamericano SOLABIAA. 7-11 April 2013, Ciudad de David, Panamá (Oral presentation).

15. **Posadas E**., Muñoz A., García-González M. C., Muñoz R., García-Encina P.A., Algal-bacterial photobioreactor for the treatment of fish farm wastewater: closing the cycle. III Congreso Latinoamericano SOLABIAA. 7-11 April 2013, Ciudad de David, Panamá (Oral presentation).

16. Muñoz Torre C.A., Laureano Casanova O., García-Encina P.A., Muñoz R., **Posadas E**, Los efectos del cambio climático en el control de la calidad del agua para la eliminación de nitrógeno y fósforo en aguas residuales agroindustriales. II Congreso Nacional de Investigación en cambio climático, 1-15 October 2012, Taumalipas (Mexico) (Oral presentation).

17. **Posadas E.**, Muñoz R., Coca M., García-González M.C., García-Encina P.A., Algal-bacterial processes for the treatment of agroindustrial wastewaters: a preliminary screening. Ecotechnologies for Wastewater Treatment: Technical, Environmental and Economic Challenges. 25-27th June 2012, Santiago de Compostela, Spain (Poster).

CO-SUPERVISION

Master Thesis

Javier Fernández Lorenzo (April 2014-July 2014). "Digested molasses treatment and biogas upgrading using algal-bacterial systems". Valladolid University (Spain).

Carmen Adriana Muñoz (April 2012-July 2012) "Control of water quality for the removal of nitrogen and phosphorous in agroindustrial wastewaters" Valladolid University (Spain).

Research Projects

Dawid Szpak (October 2012-January 2013) "Enhancing microalgae lipid content by nutrient depletion in a biogas upgrading photobioreactor" Valladolid University (Spain).

Anna Soltau (February 2012) "Carbon and nutrient removal from wastewater using microalgae-bacteria symbiotic interaction" Valladolid University (Spain).

Sonia Bochon (September 2011-January 2012) "Algal-Bacterial Processes for the treatment of Agroindustrial Wastewaters" Valladolid University (Spain).

Teaching

Environmental and Process Technology (2014). Assistant Professor (2 ECTS). Industrial Engineering Degree. 1st Course. Valladolid University (Spain). Cord Course.

Environmental and Process Technology (2015). Assistant Professor (2 ECTS). Industrial Engineering Degree. 1st Course. Valladolid University (Spain). Cord Course.

Stays abroad

School of Engineering and Advanced Technology, Massey University (Palmerston North, New Zealand): August 2015 - December 2015 - Guest PhD student.

Department of Chemical Engineering, Almería University (Almería, Spain): July 2013 – December 2013 - Guest PhD student.

Department of Environmental Technology, Tampere University of Applied Sciences (Tampere, Finland): August 2010- February 2011 – Eramus student.

Attended Short-Courses and Seminars

Culture of microalgae (by Gabriel A. Fernández), Department of Chemical Engineering and Environmental Technology, University of Valladolid (Valladolid, Spain), November 2014 (10 hours).

EMBS 2014: Euromediterranean Microalgal Biotechnology Seminar & Worskshop. 20-24 October 2014, Almería (Spain) (40 hours).

XXI Edition of Chemical Engineering Conferences (Almería University, Spain): 11-13 September 2013 (20 hours).

Producción de Biodiesel a partir de microalgas (Chiriquí, Panamá): 7 April 2013 (III Congreso Latinoamericano SOLABIAA) (8 hours).

Environmental Biotechnology, Department of Chemical Engineering and Environmental Technology, University of Valladolid (Valladolid, Spain) Academic year: 2011/2012 (25 hours).

4th Summer School Model-Based Design and Control of Waste Water Treatment Plants (San Sebastian, Spain): 27 June 2011- 1 July 2011 (40 hours).

Awards

First prize for the best paper "Impact on biomass composition during the optimization of the integral biogas upgrading in microalgal-bacterial processes" at IWA Water and Industry 2015, Innovation and Solutions for Industrial Water and Wastewater during IWA 2015, 7-10 June 2015, Vasteras (Sweden).

First prize for the best Poster "Microalgae-based processes applied to the simultaneous treatment of domestic wastewater and CO_2 removal from flue gas" in Chemical Engineering

category during II-Mini Simposio en Investigación en Ciencias Experimentales (Almería University, Spain), 15 November 2013.

First prize for the best Master Thesis 2012 in the Program in Process and Systems Engineering.

Other merits

Participation in Research & Development Projects:

- Estudio de la biodegradación de materia orgánica y recuperación de nutrientes a partir de aguas residuales de origen agroalimentario mediante sistemas simbióticos microalgas-bacterias (March 2011 - April 2013).
- CENIT VIDA: INVESTIGACIÓN EN TECNOLOGÍAS AVANZADAS PARA LA VALORACIÓN INTEGRAL DE ALGAS: Fotobiorreactores de algas (June 2011- May 2014).
- Aprovechamiento de nutrientes de efluentes agroalimentarios mediante crecimiento y valorización de biomasa algal. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) (November 2014-November 2017).
- Valorización de residuos agroalimentarios generando bioenergía y bioproductos en procesos con microalgas (November 2014-November 2017).

- Member of the Organizing Committee of the International Water Association Specialist Conference: Water and Industry 2011, 1-4 May 2011 (Valladolid, Spain).

- Participation in EDPR University Challenge 2015 under the supervision of Dr. Raquel Lebrero.