Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

Photosynthetic biodegradation of domestic and agroindustrial wastewater

Presentada por **Dimas Alberto García Guzmán** para optar al grado de Doctor por la Universidad de Valladolid

Dirigida por:

Dr. Raúl Muñoz Torre

Dra. Silvia Bolado

Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

Biodegradación fotosintética de aguas residuales domésticas y agroindustriales

Presentada por **Dimas Alberto García Guzmán** para optar al grado de
Doctor por la Universidad de Valladolid

Dirigida por:

Dr. Raúl Muñoz Torre

Dra. Silvia Bolado

Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

Memoria para optar al grado de Doctor
presentada por el Lic. en Química:

Dimas Alberto García Guzmán

Siendo tutores en la Universidad de Valladolid:

Dr. Raúl Muñoz Torre

Dra. Silvia Bolado

Valladolid, septiembre del 2018

Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

Secretaría

La presente tesis queda registrada en el

folio número _____ del correspondiente libro

de registro número _____.

Valladolid _____de _____2018

Fdo. El encargado del registro

Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

Impreso 1T

AUTORIZACIÓN DEL DIRECTOR/A DE TESIS

(Art. 7.2 de la Normativa para la presentación y defensa de la Tesis Doctoral en la UVa)

D./Dª Raúl Muñoz Torre ....., con D.N.I./Pasaporte 16811991-A.....

Profesor/a del departamento de Departamento de Ingeniería Química y Tecnología Ambiental

.....

Centro Escuela de Ingeniería Industriales (Sede Dr. Mergelina) .....

Dirección a efecto de notificaciones Calle Prado de la Magdalena 5, 47011, Valladolid .....

.....

e-mail mutora@iq.uva.es.....

como Director(a) de la Tesis Doctoral titulada "Photosynthetic biodegradation of domestic and agroindustrial wastewater" .....

realizada por D./Dª Dimas Alberto García Guzmán .....

alumno/a del Programa de Doctorado Ingeniería Química y Ambiental..... .....

....

autoriza su presentación, considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresan su conformidad con dicha presentación. La tesis se presenta por compendio de publicaciones con 3 papers Q1 publicados y uno en evaluación.

.....

.....

Valladolid, 17 de septiembre de 2018

El/La Director/a de la Tesis,

Fdo.: .....

SR/SRA. PRESIDENTE/A DE LA COMISIÓN DE DOCTORADO

Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

Impreso 1T

AUTORIZACIÓN DEL DIRECTOR/A DE TESIS

(Art. 7.2 de la Normativa para la presentación y defensa de la Tesis Doctoral en la UVa)

D./Dª Silvia Bolado Rodríguez, con D.N.I. 13924581-N

Profesor/a del departamento de Departamento de Ingeniería Química y Tecnología Ambiental

Centro Escuela de Ingeniería Industriales (Sede Dr. Mergelina)

Dirección a efecto de notificaciones Calle Prado de la Magdalena 5, 47011, Valladolid

e-mail silvia@iq.uva.es

como Director(a) de la Tesis Doctoral titulada "Photosynthetic biodegradation of domestic and agroindustrial wastewater" realizada por D Dimas Alberto García Guzmán alumno del Programa de Doctorado Ingeniería Química y Ambiental

autoriza su presentación, considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresan su conformidad con dicha presentación. La Tesis se presenta por compendio de publicaciones con 3 artículos Q1 publicados y uno en evaluación

Valladolid, 17 de septiembre de 2018

El/La Director/a de la Tesis,

BOLADO RODRIGUEZ SILVIA - DNI 13924580N

Firmado digitalmente por BOLADO RODRIGUEZ SILVIA - DNI 13924580N
Nombre de reconocimiento (DN): c=ES, o=UNIVERSIDAD DE VALLADOLID, ou=CERTIFICADO ELECTRONICO DE EMPLEADO PUBLICO, serialNumber=IDCES-13924580N, sn=BOLADO RODRIGUEZ, givenName=SILVIA, cn=BOLADO RODRIGUEZ SILVIA - DNI 13924580N
Fecha: 2018.09.17 15:23:32 +02'00'

Fdo. Silvia Bolado Rodríguez

SR/SRA. PRESIDENTE/A DE LA COMISIÓN DE DOCTORADO

Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

Reunido el tribunal que ha juzgado la Tesis Doctoral Titulada

"Photosynthetic biodegradation of domestic and agroindustrial wastewater",

presentada por el Lic. Dimas Alberto García Guzmán 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 _____2018

PRESIDENTE SECRETARIO VOCAL

Table of contents

Resumen

El incremento poblacional ocurrido en las pasadas décadas a nivel mundial ha generado una creciente demanda de alimentos y suministros, lo que ha causado un incremento irracional en el uso de los recursos hídricos. Esto ha generado grandes volúmenes de aguas residuales domésticas e industriales que requieren ser tratadas. Aunque todavía se utilizan tecnologías físico-químicas para el tratamiento de aguas residuales, sus altos costes de operación e impactos ambientales han fomentado el desarrollo de procesos biológicos. Sin embargo, el bajo potencial de los procesos de lodos activos para la recuperación de recursos de las aguas residuales junto con su alta demanda de energía, y el bajo rendimiento de eliminación de nutrientes de los procesos anaerobios, todavía hacen necesario el desarrollo de soluciones más sostenibles y competitivas para la gestión de aguas residuales domésticas e industriales. Con el objetivo de proponer nuevas soluciones de tratamiento de aguas residuales sostenibles ambientalmente y de bajo costo, esta tesis se centró en determinar el potencial y las limitaciones de los procesos fotosintéticos de depuración antes futuro escalado.

El estado del arte del tratamiento de aguas residuales mediante procesos fotosintéticos se presenta en la sección **Introduction**. Los objetivos, el desarrollo y las estrategias seguidas en esta tesis se resumen en la sección **Aims and Scope**.

En el Capítulo 1 (**Chapter 1**) se estudió la biodegradabilidad de un agua residual doméstica real (RDWW) en un nuevo fotobiorreactor anóxico-aerobio de algas y bacterias. Esta agua residual se eligió por su relevancia ambiental y baja relación C/N/P. Con el fin de superar esta limitación de carbono inorgánico, se acopló una columna de absorción de biogás al fotobiorreactor para proporcionar CO_2 adicional. El lavado de biogás permitió una casi completa nitrificación del NH_4^+ a NO_3^- y promovió un mayor crecimiento de microalgas (con la consiguiente mejora en la asimilación de N y P). Durante los 208 días de operación del sistema, las eficiencias de remoción (RE) de COT, NT, NH_4^+ y PT representaron 88, 82, 98 y 61%, respectivamente, mientras que la concentración de biomasa del efluente promedió 26 mg SST/L. Por lo tanto, los resultados aquí obtenidos confirmaron que la nueva configuración de fotobiorreactor de algas y bacterias aquí evaluada permite obtener unas adecuadas eficacias de eliminación de C, N y P, y la suplementación de biogás permitió superar la limitación de carbono inorgánico y soportó un proceso eficiente de nitrificación-desnitrificación. Además de la eliminación

de CO_2, el sulfuro de hidrógeno presente en el biogás se oxidó a sulfato utilizando el O_2 fotosintéticamente producido en el fotobiorreactor, lo que conllevó una purificación efectiva del biogás a biometano. El suministro de biogás también permitió mantener un pH estable y neutro en el fotobiorreactor, evitando así el uso de reactivos químicos costosos. Finalmente, la sedimentación y recirculación de biomasa mantuvo una concentración de biomasa estable y alta en el proceso, lo que probablemente permitió el dominio de un consorcio de *Scenedesmus* con buenas propiedades de sedimentación.

En el Capítulo 2 (**Chapter 2**) se investigó el predominio a largo plazo de las poblaciones de microalgas. La mayoría de los estudios realizados en condiciones de laboratorio o exteriores se han centrado en la eliminación de contaminantes claves presentes en las aguas residuales, prestándose muy poca atención a la monitorización de las dinámicas de población de microalgas. En este estudio, se evaluó la evolución de las poblaciones de microalgas durante el tratamiento de purines de cerdo (PWW) diluidos al 15% en cuatro fotobiorreactores abiertos operados a un tiempo de retención hidráulico (HRT) de 27 días y pH = 8 (controlado por adición de CO_2), e inoculados con *Chlorella* sp. (R1), *Acutodesmus obliquus* (R2), *Oscillatoria* sp. (R3) y en ausencia de inóculo (R4) durante 180 días. La ausencia de cultivos monoalgales en los cuatro fotobiorreactores durante el período experimental y las rápidas variaciones registradas en la estructura de las poblaciones de microalgas (principalmente en R1 y R2) indicaron la dificultad de mantener cultivos monoalgales durante el tratamiento de PWW en sistemas abiertos. En efecto, la caracterización morfológica de las microalgas reveló que la inoculación de un fotobiorreactor con una microalga específica durante el tratamiento de PWW no garantiza su dominancia a largo plazo. El dominio de *Chlorella* sp. en los cuatro fotobiorreactores abiertos confirmó la alta tolerancia de esta microalga verde a la contaminación orgánica. Las concentraciones de biomasa en estado estacionario oscilaron entre 2445-2610 mg/L en R1-R3 y 3265 mg/L en R4 (control). No se observaron diferencias significativas en las eficiencias de eliminación de COT (86-87%), CI (62-71%), NT (82-85%) y PT (90-92%). Además, las eficiencias de eliminación de Zn ascendieron a 26% en R3, 37% en R2 y 49% en R1 y R4. En general, se registró una biodegradación eficiente independientemente de las especies de microalgas inoculadas, lo que confirma la robustez de los procesos de algas y bacterias dedicados a la eliminación de carbono y nutrientes de aguas residuales agroindustriales.

El consorcio de *Chlorella* sp. obtenido en el Capítulo 2 se utilizó como inóculo en el Capítulo 3 (**Chapter 3**), donde se llevó a cabo una evaluación sistemática del potencial de los fotobiorreactores abiertos de algas-bacterias para el tratamiento de PWW en condiciones interiores y exteriores. Se evaluaron las RE de materia orgánica, nutrientes y zinc de PWW, la dinámica de la estructura de microalgas y la concentración de biomasa. Se evaluó el rendimiento de cuatro fotobiorreactores abiertos alga-bacteria operados a ≈ 26 días de HRT con PWW diluido 10 (×10) y 20 (×20) veces en ambiente interior y exterior durante 120 días. Los valores más altos de COT-RE, PT-RE y Zn-RE (94±1%, 100% y 83±2%, respectivamente) se registraron en condiciones de interior con una dilución de ×10 PWW, mientras que la NT-RE más alta (72±8%) se obtuvo en condiciones de exterior también en ×10 PWW. *C. vulgaris* fue la especie de microalga dominante, independientemente de las condiciones ambientales y de la dilución del PWW. Finalmente, el análisis de secuenciación DGGE de las comunidades bacterianas reveló la presencia de cuatro filos, siendo *Proteobacteria* el filo dominante con 15 de las 23 bandas más intensas. En resumen, este trabajo demostró por primera vez que ni la eliminación de contaminantes ni la estructura de las comunidades de microalgas y bacterias bajo condiciones de interior se puede extrapolar directamente a fotobiorreactores operados en condiciones exteriores.

Finalmente, el Capítulo 4 (**Chapter 4**) tuvo como objetivo evaluar de forma sistemática durante 224 días el rendimiento de fotobiorreactores abiertos alga-bacteria y de bacterias púrpuras fotosintéticas (PPB) durante el tratamiento en condiciones de interior de PWW bajo iluminación artificial. Se investigó la influencia del tiempo del HRT en la eliminación de carbono, nitrógeno, fósforo y zinc, y la influencia en la estructura de la población del consorcio alga-bacteria y de PPB. Además, se investigó la influencia de la dilución del PWW en el rendimiento de biodegradación de un consorcio alga-bacteria y PPB en un sistema en lote en condiciones similares al experimento en continuo. Los resultados demostraron que el fotobiorreactor de algas y bacterias (PBR-AB) soportó las mayores eficiencias de eliminación de nitrógeno, fósforo y zinc (87±2, 91±3 y 98±1%), mientras que las remociones de carbono orgánico más altas se registraron en el fotobiorreactor de PPB (PBR-PPB) a un HRT de 10,6 días (87 ± 4%). Las mayores concentraciones de biomasa fueron registradas en PBR-AB en comparación con PBR-PPB, hecho que fue más evidente a un HRT de 10.6 días, probablemente debido a la activa asimilación fotosintética de CO_2 por parte de las microalgas. *C. vulgaris*, la especie de

microalga inoculada en PBR-AB, fue detectada en todas las etapas desde el día 7 hasta el día 224, junto con otras especies de *Chlorella*. Curiosamente, la disminución del HRT provocó en PBR-PPB la aparición de microalgas desde el día 98 en adelante. La disminución del HRT en PBR-PPB también determinó el predominio de *Firmicutes* y *Epsilonbacteriaeota* (46.7% y 19.8% de abundancia a un HRT de 4.1 días) y una disminución de la contribución de *Proteobacteria* hasta una abundancia de 12.1%. Se observó también un lavado de la especie *Rhodopseudomonas* seguido del dominio de la especie *Rhodoplanes* en PBR-PPB. Este estudio reveló que el consorcio de algas-bacterias soportó un rendimiento superior en términos de eliminación de nutrientes y zinc, aunque PBR-PPB exhibió una capacidad ligeramente superior de eliminación de materia orgánica, lo cual no se observó durante los ensayos de biodegradación de PWW en lote. En general, la disminución del HRT, en lugar del tipo de iluminación, provocó cambios significativos en la estructura de las poblaciones de microalgas y bacterias.

Los resultados obtenidos en esta tesis representan una herramienta valiosa para comprender mejor la dinámica y la estructura de las poblaciones microbianas durante el tratamiento fotosintético de aguas residuales. Estos hallazgos apoyarán la optimización de los procesos de biodegradación fotosintética para mejorar la recuperación de carbono y nutrientes de los efluentes agroindustriales a través de la maximización de la productividad de biomasa. La biomasa generada durante el tratamiento de estas aguas residuales podría utilizarse como materia prima en la producción industrial de bioproductos comerciales, como biocombustibles y biofertilizantes.

Abstract

The increase in human population occurred in the past decades has resulted in an increasing demand of food and supplies, which has caused a higher use of water resources. This has generated larger volumes of domestic and industrial wastewaters that need to be treated. Although physical/chemical technologies are still used to treat wastewater, their high operating costs and environmental impacts have fostered the development of biological processes. However, the poor resource recovery potential of activated sludge processes along with their high energy demand, and the low nutrient removal performance of anaerobic process, still require the development of more cost-competitive and sustainable solutions for the management of domestic and industrial wastewaters. In order to propose new low-cost and sustainable wastewater treatment solutions, this thesis focused on determining the potential and limitations of photosynthetic processes prior to their future scale-up.

The state-of-the-art of wastewater treatment by photosynthetic processes is presented in the **Introduction** section**.** The objectives, development and strategies followed in this thesis are summarized in **Aims and Scope** section.

In **Chapter 1** the biodegradability of a real domestic wastewater (RDWW) was studied in a novel anoxic-aerobic algal-bacterial photobioreactor. RDWW was chosen based on its environmental relevance and low C/N/P ratio. In order to overcome this carbon limitation a biogas absorption column was coupled to the photobioreactor to provide additional CO_2. Biogas scrubbing supported an almost complete nitrification of the NH_4^+ to NO_3^- and promoted microalgae growth (with the subsequent enhancement in N and P assimilation). Over the 208 days of operation, the removal efficiencies (REs) of TOC, TN, NH_4^+ and TP accounted for 88, 82, 98 and 61%, respectively, while the TSS effluent concentration averaged 26 mg/L. Therefore, the results herein obtained confirmed that the novel algal-bacterial photobioreactor configuration here evaluated supported consistent removal rates of C, N and P, and biogas supplementation overcame the inorganic carbon limitation and supported an efficient process of nitrification-denitrification. In addition to CO_2 removal, the hydrogen sulfide present in biogas was oxidized to sulfate using the O_2 photosynthetically produced in the photobioreactor, which entailed an effective biogas upgrading to biomethane. Biogas supplementation also

allowed maintaining a stable and neutral pH in the photobioreactor, thus avoiding the use of costly chemical reagents. Finally, a stable and high biomass concentration was maintained in the process via biomass settling and recirculation, which likely induced the dominance of a *Scenedesmus*-based consortium with enhanced settling properties.

The long-term dominance of microalgae was investigated in **Chapter 2**. Most studies conducted under laboratory or outdoors conditions focused on the removal of key pollutants present in wastewater, while little attention has been paid to the monitoring of the dynamics of microalgae population. The dynamics of microalgae population during the treatment of piggery wastewater (PWW) diluted at 15% in four open photobioreactors operated at a HRT of 27 days and pH =8 (controlled by CO_2 addition), and inoculated with *Chlorella sp*. (R1), *Acutodesmus obliquus* (R2), *Oscillatoria sp*. (R3) and in the absence of inoculum (R4), were evaluated for 180 days. The absence of monoalgal cultures in the four photobioreactors throughout the experimental period and the rapid variations in microalgae population structure here recorded (mainly in R1 and R2) revealed the difficulty to maintain monoalgal cultures during the treatment of PWW in open systems. Indeed, the morphological microalgae characterization revealed that inoculation of a photobioreactor during PWW treatment with a specific microalga does not guarantee its long-term dominance. The higher dominance of *Chlorella* sp. in the four open photobioreactors confirmed the high tolerance of this green microalga to organic pollution. Steady state biomass concentrations ranged from 2445-2610 mg/L in R1-R3 to 3265 mg/L in R4 (control). Interestingly, no significant differences were recorded in the removal efficiencies (REs) of TOC (86–87%), IC (62-71%), TN (82-85%) and TP (90-92%). In addition, Zn-REs accounted for 26% in R3, 37% in R2, and 49% in R1 and R4. Overall, an efficient biodegradation occurred regardless of the microalgae species inoculated, which confirmed the robustness of algal-bacterial processes devoted to carbon and nutrient removals from livestock wastewaters.

The robust *Chlorella* sp. consortium obtained in **Chapter 2** was utilized as inoculum in **Chapter 3**, where a systematic evaluation of the potential of open algal-bacterial photobioreactors for the treatment of PWW under indoor and outdoor conditions was carried out. The REs of organic matter, nutrients and zinc from PWW, along with the dynamics of biomass concentration and structure were assessed. The performance of four open algal-bacterial photobioreactors operated at ≈ 26 days of HRT with 10 (×10) and 20

(×20) times diluted PWW under indoor and outdoor conditions was evaluated for 120 days. The highest TOC-RE, TP-RE and Zn-RE (94±1%, 100% and 83±2%, respectively) were achieved indoors in ×10 PWW, while the highest TN-RE (72±8%) was recorded outdoors in ×10 PWW. *Chlorella vulgaris* was the dominant species regardless of the ambient conditions and PWW dilution. Finally, DGGE-sequencing of the bacterial community revealed the occurrence of four phyla, *Proteobacteria* being the dominant phylum with 15 out of the 23 most intense bands. In summary, this work demonstrated for the first time that neither pollutant removal nor the structure of microalgae and bacterial communities under indoor conditions can be directly extrapolated to outdoors photobioreactors.

Finally, **Chapter 4** aimed at systematically evaluating for 224 days the performance of open algal-bacterial and Purple Photosynthetic Bacteria (PPB) photobioreactors for the indoor treatment of PWW under artificial illumination. The influence of the hydraulic retention time (HRT) on the removal of carbon, nitrogen, phosphorous and zinc, and on the structure of the algal-bacterial and PPB population was investigated. In addition, the influence of PWW dilution on the biodegradation performance of an algal-bacterial consortium and PPB was assessed batchwise. The results demonstrated that the algal-bacterial photobioreactor (PBR-AB) supported the highest removal efficiencies of nitrogen, phosphorus and zinc (87±2, 91±3 and 98±1%), while the highest organic carbon removals (87±4%) were achieved in the PPB photobioreactor (PBR-PPB) at a HRT of 10.6 days. The higher biomass concentrations recorded in PBR-AB compared to PBR-PPB, which were more evident at a HRT of 10.6 days, were likely due to the active photosynthetic CO_2 assimilation by microalgae. *C. vulgaris,* the microalga species inoculated in PBR-AB, was detected in all stages from day 7 to day 224, along with other *Chlorella* species. Interestingly, the stepwise decrease in HRT in PBR-PPB induced the occurrence of microalgae from day 98 onwards. The decrease in HRT in PBR-PPB also mediated the dominance of *Firmicutes* and *Epsilonbacteraeota* (46.7 % and 19.8 % of abundance at a HRT of 4.1 days) and decreased the contribution of *Proteobacteria* to 12.1%. A wash-out of *Rhodopseudomonas* followed by the dominance of *Rhodoplanes* was observed in PBR-PPB. This study revealed that algal-bacterial consortia supported a superior performance in terms of nutrients and zinc removal, although PBR-PPB exhibited a slightly better capacity to remove organic matter, which was not observed during batch PWW biodegradation tests. Overall, the stepwise decrease in HRT, rather

than the type of illumination, caused significant changes in the structure of microalgae and bacterial population.

The results obtained in this thesis represent a valuable tool to better understand the dynamics and structure of microbial populations during photosynthetic wastewater treatment. These findings will support the optimization of photosynthetic biodegradation processes to enhance carbon and nutrient recovery from livestock effluents via maximization of biomass productivity. The biomass generated during livestock treatment could be used as a feedstock in the industrial production of commercial bioproducts such as biofuels and biofertilizers.

List of publications included in the thesis

The following publications are presented as part of the present thesis. Three of them are published in international journals indexed in ISI Web of Knowledge (Papers I, II and III). Paper IV has been submitted for publication.

Paper 1. **García, D**., Alcántara, C., Blanco, S., Pérez, R., Bolado, S., Muñoz, R., 2017. ***Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading***. Chemical Engineering Journal. 313, 424 - 434. doi:10.1016/j.cej.2016.12.054

Paper 2. **García, D**., Posadas, E., Blanco, S., Acién, G., García-Encina, P., Bolado, S., Muñoz, R., 2018. ***Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors***. Bioresource Technology Journal. 248 (Pt B), 120 -126. doi:10.1016/j.biortech.2017.06.079

Paper 3. **García, D**., Posadas, E., Grajera, C., Blanco, S., Martínez, S., Acién, G., García-Encina, P., Bolado, S., Muñoz, R., 2017. ***Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions***. Bioresource Technology Journal. 245 (Pt A), 483 - 490. doi.10.1016/j.biortech.2017.08.135

Paper 4. **García D.,** de Godos, I., Dominguez, C., Turiel, S., Bolado, S., Muñoz, R. 2018. ***A systematic comparison of the potential of a microalgal-bacterial consortium and purple phototrophic bacteria for the continuous treatment of piggery wastewater***. Submitted for puplication to Bioresource Technology (Submitted: 27/08/2018).

Contribution to the papers included in the thesis

Paper 1. In this work, I was responsible of the design, start-up and operation of the experimentation under the supervision of Dr. Raúl Muñoz. I performed the mass balance calculations, results evaluation and manuscript writing under the supervision of Dr. Cynthia Alcántara, Dr. Silvia Bolado and Dr. Raúl Muñoz. Dr. Saúl Blanco and Dr. Rebeca Pérez were responsible of the characterization of the microalgal and bacterial populations, respectively, where I contributed in the data analysis and discussion.

Paper 2. I was responsible of the design, start-up and operation of the experimental set-up under the supervision of Dr. Raúl Muñoz. I performed the mass balance calculations, results evaluation and manuscript writing under the supervision of Dr. Esther Posadas, Dr. Silvia Bolado and Dr. Raúl Muñoz. Dr. Gabriel Acién was responsible of the design and construction of the control system unit of the experimental set-up. Dr. Saúl Blanco was responsible of the characterization of the microalgal populations, where I contributed in the data analysis and discussion.

Paper 3. In this work, I was responsible of the design, start-up and operation of the experimental set-up in collaboration with MSc Eng. Carlos Grajeda under the supervision of Dr. Raúl Muñoz. I conducted the mass balance calculations, results evaluation and manuscript writing under the supervision Dr. Esther Posadas, Dr. Silvia Bolado and Dr. Raúl Muñoz. Dr. Gabriel Acién was responsible of the design and construction of the control system unit of the experimental set-up. Dr. Saúl Blanco and Dr. Sonia Martínez were responsible of the characterization of the microalgal and bacterial populations, respectively, where I contributed in the data analysis and discussion.

Paper 4. During this research, I was in charge of the design, start-up and operation of the experimental set-up in collaboration with Christian Dominguez under the supervision of Dr. Raúl Muñoz. I carried out the mass balance calculations, results evaluation and manuscript writing under the supervision of Dr. Ignacio de Godos, Dr. Silvia Bolado and Dr. Raúl Muñoz. Dr. Sara Turiel was responsible of the characterization of the microalgal populations, where I contributed in the data analysis and discussion.

Introduction

1.1 Wastewater pollution

Water is the main component of the hydrological cycle, is essential for sustainable development and it possesses a large social, economic and environmental value (Figure 1) (United Nations, 2017). Water demand worldwide is foreseen to increase in the next decades, with agricultural sector expected to be responsible for 70% of water extraction (UN WWAP, 2017). Additionally, water availability nowadays is affected by human pollution caused by the intensive agricultural, industrial and urban use.

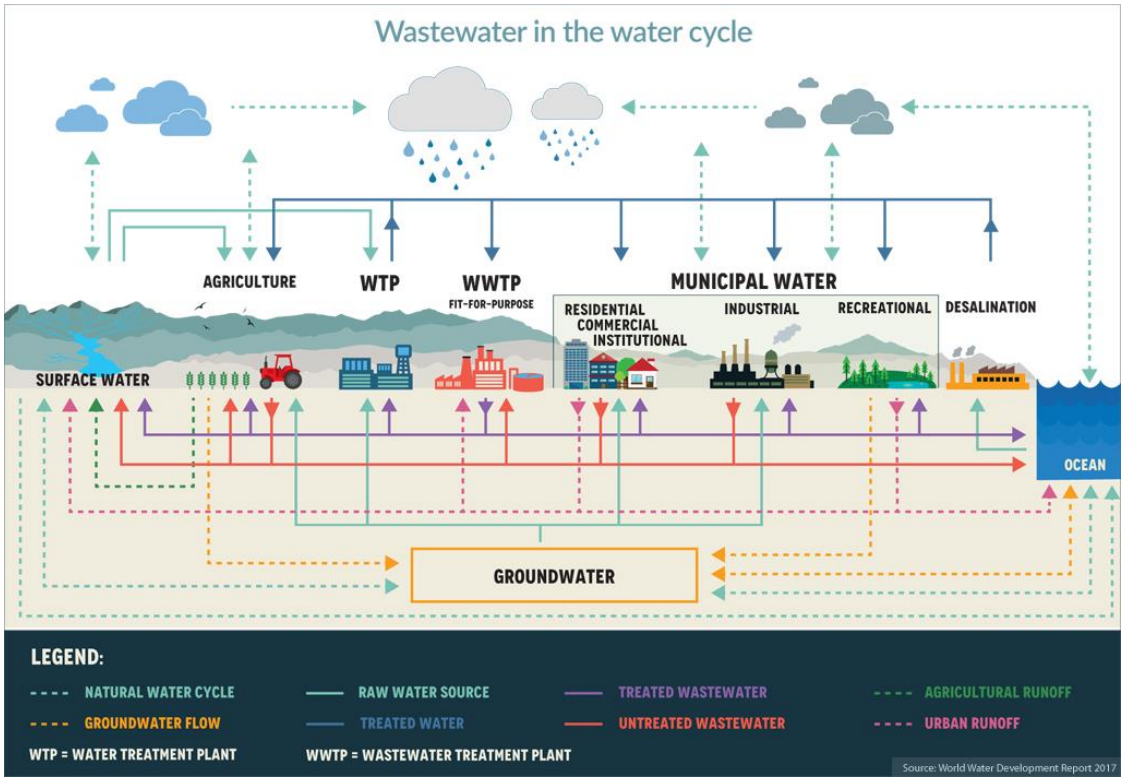


Figure 1. Wastewater in the water cycle (Source: UN WWAP, 2017).

The rapid and sustained human growth will entail a population of 11.2 billion people in 2100 (United Nations, 2015). Consequently, this increase in population density involves the increase on the use of watershed and aquifers (Ihp, 2008) and the uncontrolled discharge of nitrogen and phosphorus from human activities to water bodies (UNWater, 2015). In this context, wastewater composition has changed over the years. For instance, seventy years ago industrial activity increased the number of heavy metals and synthetic organic compounds discharged to the environment, with 10000 new organic compounds being annually introduced to natural water bodies (Metcalf and Eddy, 2003). Table 1 shows the main wastewater pollutants as a function of the type of wastewater and their associated effects.

Table 1. Main wastewater pollutants, their sources and effects.

Pollutant	Main representative parameters	Wastewater		Possible effects of the pollutant
		Domestic	Agroindustrial	
Suspended solids	Total suspended solids	xxx	↔	Aesthetic problems
				Sludge deposits
				Pollutant adsorption
				Protection of pathogens
Biodegradable organic matter	Biochemical oxygen demand	xxx	↔	Oxygen consumption
				Death of fish
				Septic conditions
Nutrients	Nitrogen, Phosphorus	xxx	↔	Eutrophication
				Toxicity to fish (ammonia)
				Illness in new-born infants (nitrate)
				Pollution of groundwater
Pathogens	Coliforms	xxx	↔	Water-borne diseases
Non-Biodegradable organic matter	Pesticides, some detergents, others	x	↔	Toxicity (various)
				Foam (detergents)
				Reduction of oxygen transfer (detergents)
				Non-biodegradability
				malodours (i.e. phenols)
Metals	Specific elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, etc.)	x	↔	Toxicity
				Inhibition of biological treatment
				Restrictions in agriculture use of sludge
				Contamination of groundwater
Inorganic dissolved solids	Total dissolved solids, conductivity	xx	↔	Excessive salinity – harm to crops (irrigation)
				Toxicity to plants (some ions)
				Problems with soil permeability (sodium)
x: small	**xx**: medium	**xxx**: large	↔ variable	**empty:** typically not important

(Source: modified from von Sperling, 2007).

The Europe Union (EU) is currently the second largest pig producer in the World after China, with an average yearly production of $154 \cdot 10^6$ pig head over the last 10 years (Figure 2) (statista, 2018). European pig farming generates 215 - $430 \cdot 10^6$ m^3/year (4-8 L/day/pig) of piggery wastewater (PWW) containing high concentrations of organic matter and nutrients (Table 2) (de Godos et al., 2009a).

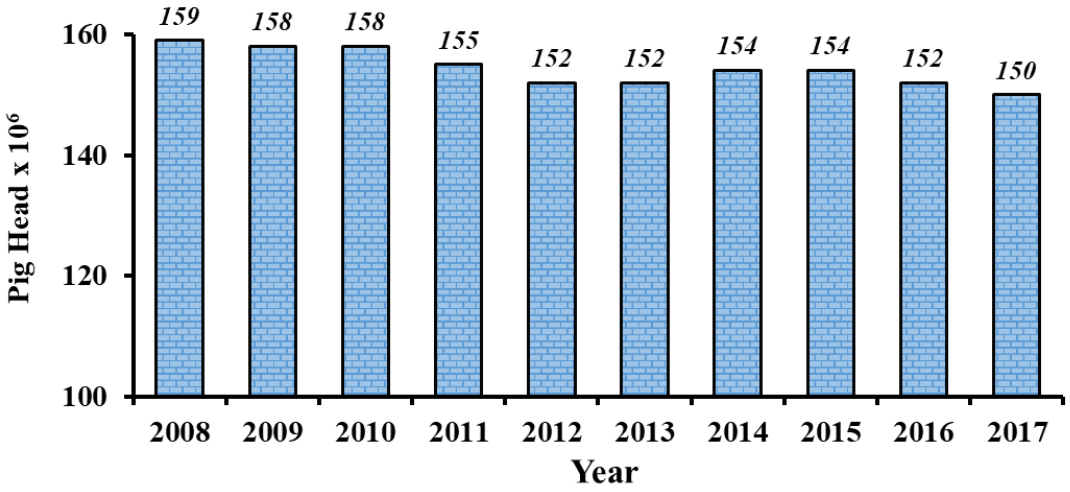


Figure 2. Pig population in European Union during the last 10 year (statista, 2018).

The estimated average organic matter and nutrient load from EU piggery wastewater in 2017 amounted to 8.923.000 tn chemical oxygen demand (COD)/year, 890.000 tn nitrogen (N)/year and 223.000 tn phosphorous (P)/year (statista, 2018). Likewise, an excessive input of residual phosphorus and nitrogen can cause eutrophication in rivers and lakes (García Ferrero, 2013; Olguín, 2003). In addition, PWW can contain heavy metals such as zinc and copper, typically used as growth promoters in swine nutrition (Abe et al., 2012; De la Torre et al., 2000). Table 2 shows piggery wastewater composition.

Table 2. Piggery wastewater composition.

Parameters	**Range (mg/L)**	
	Min	Max
Total Solid (TS)	6000	7000
Total Nitrogen (TN)	3000	4700
Total Phosphorus (TP)	900	1300
BOD_5	10000	20000
COD	38000	42000
Organic matter	35000	40000
Heavy metals (HMs)	variable	

Source: (García Ferrero, 2013).

1.2 Photosynthetic wastewater treatment

Wastewater treatment (WWT) regulations are becoming stricter worldwide to prevent the development of septic conditions, mal odors, eutrophication and toxicity in natural water bodies. Many technologies have been engineered and up-scaled in the past century to deal with the health and environmental concerns derived from the uncontrolled discharge of untreated wastewater. Among them, photosynthetic wastewater treatment (either based on microalgae or on purple photosynthetic bacteria) has emerged as a promising platform for wastewater treatment coupled with resource recovery (Metcalf and Eddy, 2003).

Algal-bacterial consortia in photobioreactors can actively reduce the levels of organic matter and nutrients in wastewater via aerobic carbon oxidation and nutrient assimilation into biomass (de Godos et al., 2014; Liu et al., 2017). Likewise, the heavy metals present in PWW are typically removed by biosorption (Abe et al., 2012; Muñoz et al., 2006; Romera et al., 2007), while photosynthesis promotes the uptake of the greenhouse gas carbon dioxide (CO_2) and supplies the oxygen required for the aerobic oxidation of organic matter (Figure 3) (Cho et al., 2013).

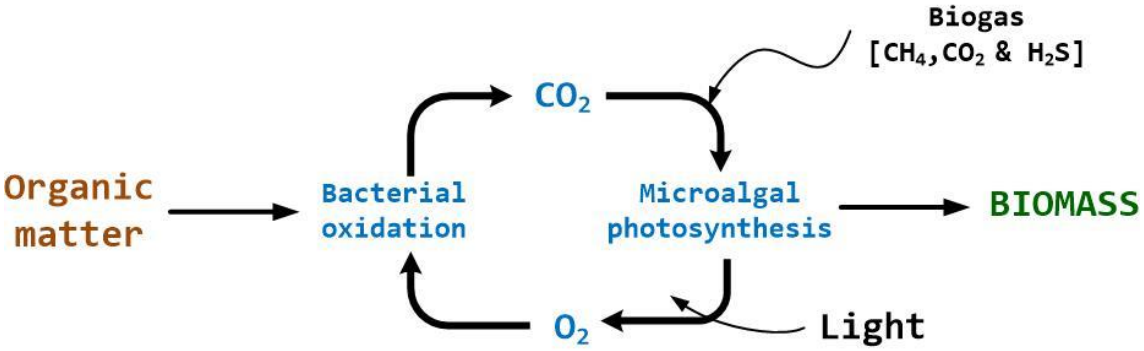


Figure 3. Algal-bacterial symbiosis during organic matter oxidation in photobioreactors. Source: (Muñoz and Guieysse, 2006).

However, microalgae metabolism requires proper physical, chemical and biological conditions in order to support an effective pollutant removal. Thus, temperature (Choi et al., 2013; Rawat et al., 2011), pH (Arbib et al., 2013) (Markou et al., 2014) and light irradiance (Cuellar-Bermudez et al., 2015; Lavoie and de la Noüe, 1985) are key environmental parameters determining microalgae growth during photobioreactor operation. Additionally, the aqueous concentrations of dissolved oxygen (DO) (Diameter and Sewers, 2000) (Muñoz and Guieysse, 2006) and carbon dioxide (Heubeck et al.,

2007; Park and Craggs, 2010; Posadas et al., 2015a) and the hydraulic retention time (HRT) (Gutiérrez et al., 2015; Hao et al., 2013; Sutherland et al., 2014) also govern the performance of microalgae during wastewater treatment in algal-bacterial photobioreactors.

For instance, Shriwastav et al., (2014) investigated the effects of nutrient levels on the growth and nutrient uptake, as well as the effects of cultivation conditions, on the physiology of *Chlorella Sorokiniana*. Evans, et al., (2017) evaluated the potential of *Chlorella vulgaris* for the treatment of municipal primary settled in term of NH_3, PO_4 and COD removal (Evans et al., 2017). Likewise, *Chlorella* sp. GD was used for the production of biomass and lipids using piggery as cultivation medium (Kuo et al., 2015). Prandini et al., (2016) used *Scenedesmus* sp. to enhance nutrient removal from swine wastewater digestate coupled to biogas upgrading in two interconnected 16.9 L glass closed-photobioreactors.

On the other hand, Purple Photosynthetic Bacteria (PPB) have the potential to become a key player in the coming generation of wastewater treatment technologies. Indeed, PPB have the ability to transform a great variety of inorganic and organic pollutant into harmless minerals or valuable products, which can be recycled. Indeed, PPB can support the bioconversion of residual organic matter into polyhydroxyalkanoate (Hassan et al., 1997), Polypoly-ß-hydroxybutyrate (Khatipov et al., 1998; Luongo et al., 2017), biohydrogen (Basak and Das, 2007) and phototrophic bacterial biomass (Azad et al., 2001). PPB have a very versatile metabolism, able to grow either in the presence or absence of molecular oxygen (O_2) (Rittmann and McCarty, 2012) and phototrophically based on infrared light (Puyol et al., 2017). PPB photosynthesis is carried out with reduced sulfur compounds or simple organic compounds as electron donor according (Figure 4). PPB can grow autotrophically using infrared light as the energy source for CO_2 fixation and inorganic electrons donor such as H_2, Fe^{2+}, S^{2-} or $S_2O_3^{2-}$ (cyclic anoxygenic photosynthesis) (Goldman et al., 1972; Overmann and Garcia-Pichel, 2013).

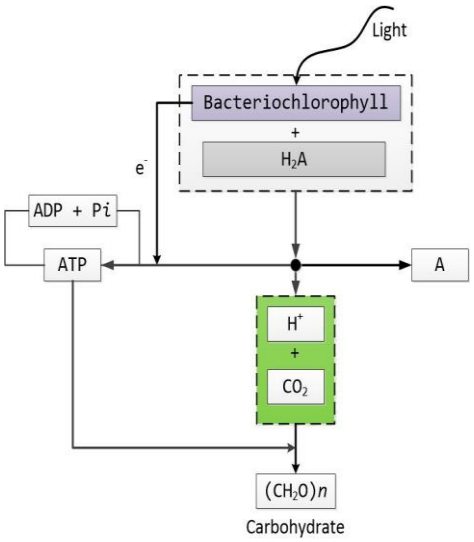


Figure 4. Mechanism of PPB photosynthesis. H_2A refers to the electron donor (Goldman et al., 1972).

Moreover, PPB can also grow heterotrophically in presence and absence of light. Figure 5 shows the schematic mechanistic model of PPB metabolism during domestic wastewater treatment (Puyol et al., 2017).

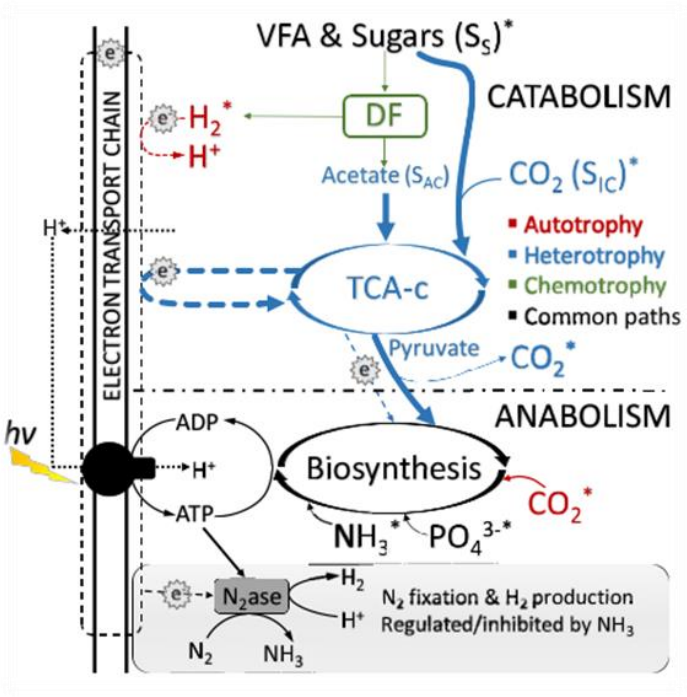


Figure 5. Schematic summary of PPB metabolism during domestic wastewater treatment. Key: N_2ase: Nitrogenase complex. TCA-c: Tri-carboxylic acid cycle. DF: Dark fermentation. VFA: volatile fatty acids. e^-: electrons. Dashed lines: electron flow. Dotted line: proton flow. S_{AC} Concentration of acetate (mg COD/L). S_{IC} Concentration of inorganic carbon (mol C/L). S_S Concentration of biodegradable soluble fraction but excluding acetate, alcohols and some sugars (mg COD/L).*: Model components. Source (Puyol et al., 2017).

1.3 Microalgae-based wastewater treatment

Algal-bacterial symbiosis constitutes a cost-effective technology for the treatment of domestic wastewater (Table 1) and piggery wastewater because of their high concentration of biodegradable organic matter and nutrients (Table 2) (de Godos et al., 2010). This technology has attracted a significant attention in the past decade based on the global quest for environmentally friendly technologies for wastewater treatment (Muñoz et al., 2012), the mitigation of greenhouse gases (Dassey and Theegala, 2013) and recent interest in biomethane production (Xia and Murphy, 2016). The lower energy demand of microalgae-based wastewater treatment compared to conventional activated sludge systems, along with the ability of microalgae to fix CO_2 phosynthetically, significantly increase the environmental sustainability of this technology (Cheah et al., 2016; Dassey and Theegala, 2013). (Unnithan et al., 2014). The *in-situ* oxygen supply mediated by microalgae photosynthesis during organic matter oxidation represents however the main advantage of this green technology (Oswald et al., 1957). Microalgae-based processes can support an effective wastewater treatment coupled to the recovery of microalgae rich in proteins, carbohydrates and lipids that can be used as a feedstock for the production of added-value products using a biorefinery approach (Olguín et al., 2013). However, despite microalgae cultivation in wastewater entails significant economic and environmental advantages over conventional CO_2-supplemented mass production of microalgae in mineral salt media, controversy still exists in literature about the possibility of maintaining monoalgal cultures during microalgae-based wastewater treatment.

As stated in section 1.2, algal growth during wastewater treatment is influenced by abiotic factors such as pH, salinity, temperature, light (quality and quantity) and the concentration of nutrients, ions, toxicants, dissolved oxygen and carbon dioxide (Gupta and Diwan, 2017). Likewise, microalgae activity is also influenced by biotic factors such as the presence of pathogens (bacteria and viruses), predation by zooplankton and competition for essential nutrients with other microorganisms (Lau et al., 1995). Finally, algal productivity depends on operational parameters such as culture agitation, HRT, addition of external inorganic carbon and harvest frequency (de la Noüe et al., 1992).

Microalgae can grow under heterotrophic and phototrophic conditions and require the supply of essential elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus,

sulfur and iron (Rittmann and McCarty, 2012). Microalgae capture sunlight during oxygenic photosynthesis, being responsible for the production of approximately half of atmospheric oxygen on earth and absorbing massive amounts of carbon dioxide (Safi et al., 2014). Carbon dioxide, whose concentration has increased from 280 to 390 ppm_v since the pre-industrial era till 2013, is the main greenhouse gas emitted nowadays (Cheah et al., 2015; Wayne Chew et al., 2017). Microalgae are able to biosequester CO_2 from flue gases generated in power plants, which can contribute to significantly reduce greenhouse gas emissions (Cheah et al., 2015). Likewise, the superior heavy metal biosorption in algal-based processes compared to conventional activated sludge processes has been recently demonstrated (Muñoz et al., 2012). Additionally, microalgae-based wastewater treatment is typically envisaged as a platform for algal biofuel feedstock production (Alcántara et al., 2015c).

Despite the above-mentioned advantages, this technology exhibits some disadvantages such as its high land requirements and the poor control of microalgae population in large-scale open systems. Likewise, microalgae-based WWT has some limitations for full-scale implementation (Muñoz et al., 2012) derived from the limited number of cost-effective biomass harvesting techniques that can cope with the poor sedimentation of most species (Su et al., 2011). Process operation at short HRT (<5 days) can limit ammonium nitrification (Alcántara et al., 2015a; Chaumont, 1993).

The mechanisms of carbon, nitrogen, phosphorous and heavy metal removal underlying WWT in algal-bacterial photobioreactors are described in the following sections.

1.3.1 Carbon Removal

Carbon in the form of carbon dioxide from the atmosphere, flue gas, biogas or organic matter biodegradation can be fixed via photosynthesis into microalgae biomass (Cai et al., 2013). Likewise, domestic, agroindustrial and livestock wastewaters contain high concentrations of organic matter and inorganic carbon that will be fixed into algal-bacterial biomass during WWT via aerobic carbon oxidation and photosynthesis. Equations 1 and 2 exemplify these reactions through the aerobic oxidation of glucose and the production of carbohydrates [1] and [2] (Rittmann and McCarty, 2012):

$$C_6H_{12}O_6 + 3\,O_2 \rightarrow 3(CH_2O)h + 3\,CO_2 + 3H_2O \quad [1]$$

The aerobic biodegradation of one mol of glucose by heterotrophs (*h*) consumes three mol of oxygen, which we can obtain by converting three mol of carbon dioxide into oxygen by microalgae according to Equation [2]. Empirical observations in our lab have consistently demonstrated that the main mechanisms of carbon removal in WWT open-photobioreactors are bioassimilation and carbon dioxide stripping (Posadas et al., 2014).

$$CO_2 + H_2O + Light \rightarrow (CH_2O)p + O_2 \quad [2]$$

Equation 2 clearly shows that the production of one mole of oxygen and one mole of phototrophic biomass (*p*) is associated to the assimilation of one mole of CO_2.

Biogas upgrading can also support CO_2 removal: In this context, biogas production by anaerobic digestion reduces the emissions of methane (CH_4) and nitrous oxide (N_2O) potentially produced during the storage and utilisation of untreated animal manure as fertiliser. The global warming potential of methane is higher than CO_2 and N_2O by factors of 23 and 296, respectively (Seadi et al., 2008). Biogas is mainly composed of methane (CH_4 ≈ 40-75%), (CO_2 ≈ 25-50%), hydrogen sulfide (H_2S ≈ 0.005-2%) and ammonia (NH_3 < 1%) (Toledo-Cervantes et al., 2017a). In this context, biogas supplementation to photobioreactors improve biomass productivity, nutrient removal and generates a high quality biomethane (Figure 6) (Posadas et al., 2017; Serejo et al., 2015). Photosynthetic biogas upgrading is based on the fixation of CO_2 via photosynthesis by microalgae and the oxidation of H_2S to sulfate by sulfur oxidizing bacteria using the oxygen photosynthetically produced (Toledo-Cervantes et al., 2016).

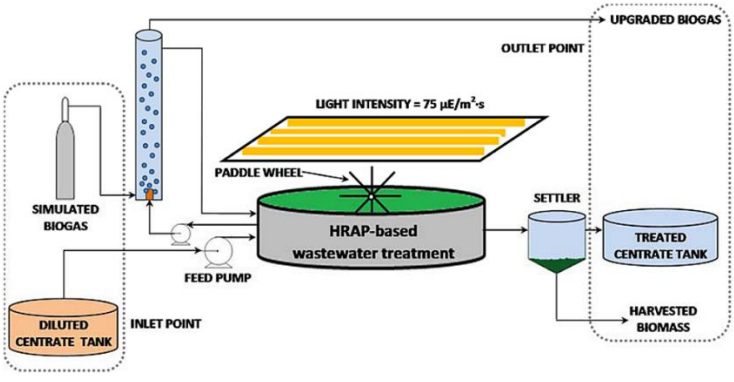


Figure 6. Schematic of the experimental set-up devoted to the simultaneous biogas upgrading and diluted centrate treatment (Source: Alcántara et al., 2015b)**.**

According to the Directive 2003/55/EC (European Parliament, 2003), the upgraded biogas must comply with specific quality requirements to be injected into natural gas grid:

of $CH_4 \geq 95\%$, $CO_2 \leq 2\%$, $O_2 \leq 0.3\%$ and negligible amounts of H_2S. Table 3 compiles the composition of biomethane obtained in microalgae-based processes.

Table 3. Compilation of the composition of the biomethane produced in microalgae-based upgrading processes.

Type of Biogas	Type of PBR	L/G ratio	CO_2 (%)	O_2 (%)	N_2 (%)	CH_4 (%)	References
+Raw	20L Bag	-	N.R	N.R	N.R	88.25	(Xu et al., 2015)
*Synthetic	HRAP+AC	1	0.4	0.03	2.4	97.2	(Toledo-Cervantes et al., 2016)
*Synthetic	HRAP+AC	0.5	0.4	0.7	2.7	96.2	(Toledo-Cervantes et al., 2017b)
*Synthetic	HRAP+AC	1	N.R	N.R	N.R	99.6	(Marín et al., 2018)

*Synthetic biogas CH_4:CO_2:H_2S=70%:29.5%:0.5%
+Raw biogas CH_4:CO_2:H_2S= 58.67±3.45%:37.54± 2.93%:0.79±0.06%
N.R- Not reported.
AC: absorption column

1.3.2 Nutrient removal

Microorganisms typically prefer ammonium over nitrate and nitrite as a nitrogen source for cells synthesis based on its lower oxidation state, although when ammonium is not available many prokaryotic cells can use oxidized forms of nitrogen to support protein synthesis (Rittmann and McCarty, 2012) (Figure 7). Nitrogen in the form of NH_4^+ and organic nitrogen is typically present at high concentrations in domestic and livestock wastewaters, while the presence of nitrate in industrial discharges. Several mechanisms, ranging from bioassimilation to abiotic processes, support nitrogen removals of 90-98% during microalgae-based wastewater treatment (Alcántara et al., 2015b).

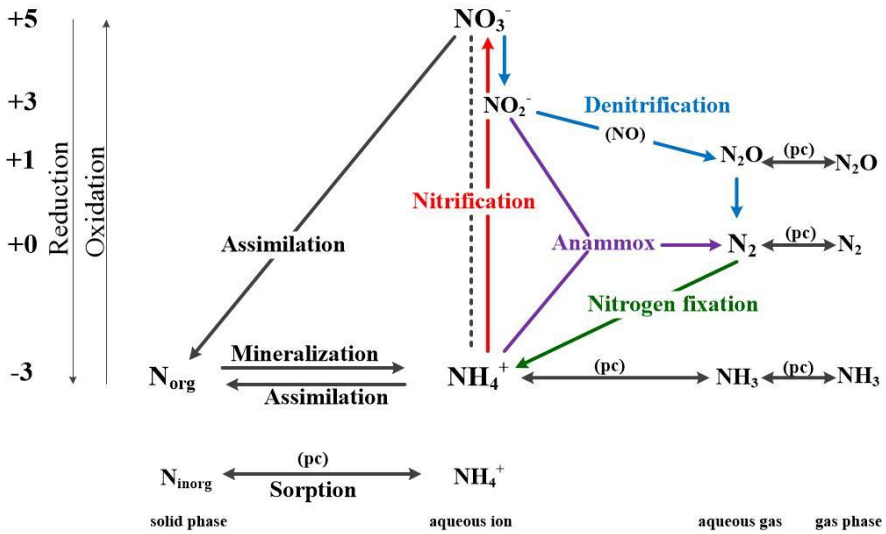


Figure 7. Biogeochemical and physical-chemical (pc) processes underlying the speciation of nitrogen in aquatic systems. (Source: Tsuyuzaki, 2014).

1.3.2.1 Assimilatory nitrogen removal

During microalgae-based wastewater treatment, nitrogen removal via assimilation is limited by the concentration of total carbon (organic+inorganic) present in the wastewater. Overall, biomass yields are lower when nitrate or nitrite are used as a nitrogen source than those obtained with ammonium (Alcántara et al., 2015c). Table 4 compiles the removal efficiencies of nitrogen in algal-bacterial photobioreactors treating wastewater.

Figure 8 shows the steps followed by the reduction of oxidized nitrogen to ammonium and its further incorporation into amino acids. Nitrate and nitrite reduction is carried out by the enzymes nitrate and nitrite reductase, respectively. Nitrate reductase uses the reduced form of nicotinamide adenine dinucleotide (NADH) to transfer two electrons, resulting in the conversion of nitrate into nitrite. Nitrite is reduced to ammonium by the enzyme nitrite reductase and ferrodoxin (Fd). All forms of inorganic nitrogen are ultimately reduced to ammonium prior to their assimilation into aminoacids within the cytoplasm. Finally, glutamate (Glu), adenosine triphosphate (ATP) and the enzyme glutamine synthase facilitate the incorporation of ammonium into the amino acid L-glutamine.

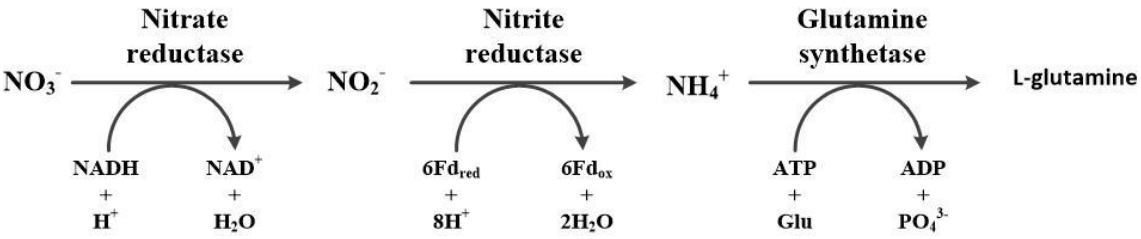


Figure 8. Simplified schematic of the assimilation of nitrate. (Source: Cai et al., 2013).

1.3.2.2 Dissimilatory nitrogen removal

More specifically, nitrification is carried out by nitrifying bacteria in a two-step process (Rittmann and McCarty, 2012). *Nitrosomonas* carry out the first step where NH_4^+ is oxidized to NO_2^- according to the energy-yielding reaction depicted (equation [3]). Species from the genera *Nitrosococcus*, *Nitrosopira*, *Nitrosovibrio* and *Nitrosolobus* are also able to oxidize NH_4^+ to NO_2^-.

$$\frac{1}{6}NH_4^+ + \frac{1}{4}O_2 = \frac{1}{6}NO_2^- + \frac{1}{3}H^+ + \frac{1}{6}H_2O \quad [3]$$

The second step of nitrification is the aerobic oxidation of NO_2^- to NO_3^- (equation [4]),

which is typically carried out by the genera *Nitrobacter* and *Nitrospira*.

$$\frac{1}{2}NO_2^- + \frac{1}{4}O_2 = \frac{1}{2}NO_3^- \quad [4]$$

On the other hand, denitrification consists of the dissimilatory reduction of NO_3^- or NO_2^- to N_2, where NO_3^- or NO_2^- are the electron acceptors for energy generation during the oxidation of organic matter or reduced inorganic compounds. The denitrification process occurs in four sequential steps where NO_3^- is reduced first to nitrite NO_2^- (equation [5]), then nitric oxide (NO) (equation [6]), nitrous oxide (N_2O) (equation [7]) and finally to N_2 gas (equation [8]).

$NO_3^- + 2e^- + 2H^+ = NO_2^- + H_2O$ Nitrate reductase [5]

$NO_2^- + e^- + 2H^+ = NO + H_2O$ Nitrite reductase [6]

$2\,NO + 2e^- + 2H^+ = N_2O + H_2O$ Nitric oxide reductase [7]

$N_2O + 2e^- + 2H^+ = N_{2(g)} + H_2O$ Nitrous oxide reductase [8]

Overall, the reduction from NO_3^- to N_2 requires 10 electron equivalents per mol of nitrogen (N_2): four electrons in the first step and two electron in the following three steps (Rittmann and McCarty, 2012). During wastewater treatment, nitrification and denitrification processes can occur either simultaneously in the same tank or in two separate tanks. In the particular case of microalgae-based wastewater treatment, several studies have consistently shown the feasibility to conduct denitrification in an anoxic tank interconnected via an internal recirculation to a photobioreactor supporting NH_4^+ oxidation (Alcántara et al., 2015a; de Godos et al., 2014).

1.3.2.3 Abiotic nitrogen removal

Microalgae photosynthesis increases the pH in the cultivation broth as a result, among others, of CO_2 uptake (equation [9]). This rise in the pH is severe under carbon limiting conditions (Alcántara et al., 2015c). This pH increase mediated by algal photosynthesis results in an enhanced NH_4^+ stripping (in the form of NH_3 with is the dominant form at high pH). Thus, abiotic nitrogen removal in open ponds is controlled by parameters that determine algae activity solar radiation, temperature, HRT or sludge retention time, agitation and superficial area (García et al., 2000). Table 4 compiles the removal efficiencies of nitrogen in algal-bacterial photobioreactors treating wastewater.

$CO_2\,(l) + H_2O\,(l) \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2\,H^+$.....[9]

Table 4. Compilation of removal efficiencies of nitrogen and phosphorus in algal-bacterial photobioreactors treating wastewater.

Type of Wastewater	Type of PBR	Volumen (L)	HRT (days)	TN (%)	TP (%)	References
Piggery	HRAP	464	10	88	*	(de Godos et al., 2009a)
Piggery	Tubular biofilm	7.5	7	100	90	(de Godos et al., 2009b)
Piggery	Enclosed jacketed glass	3.5	4.4	37	*	(de Godos et al., 2010)
Piggery	HRAP	400	40	86	*	(Aguirre et al., 2011)
Fish processing	HRAP	3	5	> 95	73	(Riaño et al., 2011)
Fish processing	HRAP	3	10	> 99	76	(Riaño et al., 2012)
Centrate & domestic	Biofilm	31	10	70	85	(Posadas et al., 2013)
Raw domestic	Biofilm	31	10	92	96	(Posadas et al., 2014a)
Fish farm & domestic	HRAP	180	10	83	94	(Posadas et al., 2014b)
Domestic	Raceway	800	6	98	6	(Posadas et al., 2015a)
Vinasse wastewater	HRAP	180	7	74	78	(Posadas et al., 2015b)
Synthetic wastewater	Enclosed jacketed glass	3.5	2	79	*	(Alcántara et al., 2015a)
Piggery	Vertical column	4	4	99	94	(Lee and Han, 2016)
Centrate	HRAP	180	4	86	92	(Posadas et al., 2017)

*- Not determined

1.3.3 Phosphorus removal

1.3.3.1 Biological phosphorus removal

Phosphorus is an essential macronutrient that spurs the growth of microalgae and cyanobacteria, leading to accelerated eutrophication of lakes (Rittmann and McCarty, 2012). Table 4 compiles the removal efficiencies of phosphorus in algal-bacterial photobioreactors treating wastewater. There are two biotic mechanisms to remove phosphorus in algal-bacterial processes devoted to WWT (Rittmann and McCarty, 2012).

a.) Regular phosphorous uptake into biomass: during regular phosphorus uptake the algal-bacterial biomass typically contains 0.5 to 1% P dry weight basis. This phosphorous is used to build-up cell membranes and genetic material.

b.) Enhanced biological phosphorus uptake: Certain heterotrophic bacteria present in the algal-bacterial mixed liquor during WWT are capable of sequestering high levels of phosphorus as intracellular polyphosphate (poly P), which is an energy storage material. These bacteria are collectively called polyphosphate accumulating organisms (PAOs), the most well-known group of PAOs being '*Candidatus Accumulibacter phosphatis*' (Yuan et al., 2012). If such microorganisms are selected, induced to store poly P, and wasted when rich in poly P, the net removal of P through biomass uptake can be increased significantly. When the enhanced biological phosphorus removal is successful, the biomass wasted can contain up to 2 to 5 times higher P content than regular biomass (Rittmann and McCarty, 2012).

1.3.3.2 Abiotic phosphorus removal

As indicated above, the microalgae photosynthesis shifts the equilibrium represented by equation [9], increasing the pH and dissolved oxygen (DO) levels in the cultivation broth. Phosphate precipitation occurs at high pH in the presence of Ca^{+2} and Mg^{+2} via hydroxyapatite formation and surface adsorption via formation of hydrogen bonds with the extracellular polysaccharides secreted by microalgae (equation [10]) (González-Fernández and Muñoz, 2017) (Liu et al., 2017).

$$3\,HPO_4^{2-} + 5\,Ca^{2+} + 4\,OH^- \rightarrow Ca_5(OH)(PO_4)_3 + 3\,H_2O \quad \text{.....[10]}$$

1.3.4 Heavy metals removal

Microalgae support heavy metals removal from wastewater through a combination of passive and active mechanisms. In this context, the main mechanisms of metal uptake in microalgae are physical adsorption, ion exchange, transport across microbial cell wall and bioassimilation, complexation and precipitation (Javanbakht et al., 2014). (Chojnacka et al., 2005). During WWT in algal-bacterial photobioreactors, bacterial biosorption also contributes to HMs removal (Mohamed, 2015). Table 5 compiles the removal efficiencies of Zn (heavy metal typically used in pig nutrition) by microalgae during WWT.

Table 5. Compilation of the removal efficiencies of Zn by microalgae.

Type of Wastewater	Type of PBR	Algae type	Zn initial concentration (mg/L)	Zn removal (%)	References
Acid wastewater	Batch test	*Cyanidium caldarium*	41.5	95	(Ahlf, 1988)
Zinc ore treatment plant	Batch test	*Oscillatoria anguistissima*	2.2 8.5 75.0	66 93 92	(Ahuja et al., 1999)
Zn Solutions	Batch test	*Scenedesmus obliquus*	0.5 1.5 4.5 8.0	59 52 45 41	(Omar, 2002)
		Scenedesmus quadricauda	0.5 1.5 4.5 8.0	61 49 43 37	
Synthetic wastewater & real mine dump leachate	Porous Substrate Bioreactor (PSBR)	*Stichococcus bacillaris*	2.0 3.0	80 80	(Li et al., 2015)
Zn Solutions	Batch test	*Chlorella minutissima* UTEX234*1*	2 (mM) 4 (mM) 6 (mM)	62 46 38	(Yang et al., 2015)
Zn Solution	Batch test	*Chlorophyceae spp*	3.0	88	(Saavedra et al., 2018)

1.4 Purple Phototrophic Bacteria-based wastewater treatment

Purple phototrophic bacteria (PPB) are ubiquitous in fresh and marine water, soil, wastewater and activated sludge (Myung et al., 2004). PPB can use multiple sources of energy and carbon for growth and maintenance. For instance, PPB can utilize different type of carbon as a carbon source for the synthesis of new cellular material, heterotrophic microorganisms being able to utilize organic carbon as an energy source while autotrophic organisms utilize carbon dioxide exclusively as a carbon source (United States Environmental Protection Agency, 2013). PPB are classified according to their ability to use infrared light as the energy source as phototroph and chemotrophs (Overmann and García-Pichel, 2013). In addition, PPB require nutrients and trace metals for cell synthesis (Rittmann and McCarty, 2012). PPB-based WWT processes have emerged as an alternative to conventional wastewater treatment with a superior potential for energy and nutrient recovery (Hülsen et al., 2016b). In this context, PPB have attracted recent interest due to their capacity to transform organic matter and nutrients with high substrate-to-product conversion yields, in the absence of oxygen, and under a wide range of infrared light wavelengths (Basak and Das, 2007; Hülsen et al., 2016b; Puyol et al., 2017). For instance, the performance of PPB has been tested for the treatment of domestic

(Hülsen et al., 2016a, 2016b, 2014; Zhang et al., 2003), piggery (Myung et al., 2004), rubber sheet (Kantachote et al., 2005), pharmaceutical (Madukasi et al., 2010), palm oil mill (Hassan et al., 1997) and fishery wastewaters (de Lima et al., 2011). PPB are considered a versatile platform for WWT based on their high grow rates, ability to assimilate organic matter and to remove nutrients by denitrification and poly-P formation (Hülsen et al., 2014). The recovery of organic matter and nutrients from WW is typically carried out in a photobioreactor that utilizes infrared light as a source for PPB growth.

The next sections describe the main mechanisms of carbon, nutrient and heavy metal removal by PPB.

1.4.1 Carbon removal

The main characteristic of PPB is their high metabolic versatility. Indeed, these microorganisms are unique in their ability to employ all known modes of metabolism to assimilate carbon (Figure 5) (Puyol et al., 2017). In addition to the obvious function of providing carbon for cell material construction, CO_2 assimilation and organic carbon oxidation play a central role in maintaining the redox balance of PPB (Hunter et al., 2009). On the other hand it should be stressed that although PPB possess simplified, and sometimes unique, photosystems compared to higher plants and green algae, their carbon metabolism pathways are rather complex (Tang et al., 2011).

1.4.1.1 Autotrophic CO_2 fixation

Photosynthesis supports the assimilation of CO_2 autotrophically into cellular material with the aid of light, with the most well-known CO_2 assimilation (Tang et al., 2011). Many metabolites, intermediates or products produced by these autotrophic carbon fixation pathways are essential for building cellular material (Hügler and Sievert, 2011; Tang et al., 2011).

1.4.1.2 Carbohydrate metabolism

Most heterotrophic organisms use carbohydrates as a carbon sources to build up cell material and provide reductive power. Three are carbohydrate catabolic pathways in photosynthetic organisms: the Emden–Meyerhof–Parnas (EMP) pathway (glycolysis), Entner–Doudoroff (ED) pathway, and PP pathway (phosphogluconate pathway) (Tang et al., 2011).

1.4.2 Biological nutrient removal

PPB can assimilate NH_4^+ and reduce nitrate to organic N for assimilatory purposes. The genes for nitrate assimilation are expressed when ammonia and other forms of fixed nitrogen are limiting and nitrate is available. PPB are also able to fix N_2 (Hunter et al., 2009). The three main mechanism involved in nitrogen removal are reviewed in the following sections.

1.4.2.1 Nitrogen assimilation

Purple phototrophic bacteria have the ability to reduce nitrate to ammonium for assimilatory purpose. This is an 8-electron reduction step where nitrate is reduced to nitrite and nitrite is further reduced to ammonium (Figures 7, 8). See section 1.3.2.1

1.4.2.2 Denitrification

Denitrification is the biological reduction of nitrate to nitrite, nitric oxide, nitrous oxide and finally to gaseous nitrogen oxide using an electron donor (Dworkin et al., 2006). There are four enzymes required for the generation of N_2 from nitrate, which entail the production of three intermediates: nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) (Figure 7). Therefore, denitrification involves the enzymes nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos). For instance, the genomes of the three *Rhodobacter sphaeroides* and five *Rhodopseudomonas palustris* strains available in literature clearly show the variability in the distribution of denitrification machinery among photosynthetic denitrifiers, the phylogenetic group with the largest number of denitrifiers being proteobacteria with denitrification being particularly prominent among α-proteobacteria (Dworkin et al., 2006).

1.4.2.3 Nitrogen Fixation

The large reservoir of nitrogen gas in our atmosphere is made biologically available by diazotrophic (N_2-fixing) bacteria as a result of its ability to reduce dinitrogen to ammonium. The ability to fix dinitrogen is found only among representatives of two of the primary kingdoms of living organisms: prokaryotic archaea and bacteria. The reduction of nitrogen gas to ammonium requires 8 electrons as shown in equation [11] (Hunter et al., 2009).

$$N_2 + 8e^- + 8H^+ + 16 - 24\,ATP \rightarrow 2NH_3 + 12H_2 + 16 - 24\,ADP + 24\,P_i \qquad [11]$$

The reduction of N_2 to NH_3 is catalyzed by enzyme called nitrogenasea, and the genes required for nitrogen fixation are commonly called nif genes. Nitrogenases consist of two dissociable metalloproteins, dinitrogenase (e.g., NifDK) and dinitrogenase reductase (e.g., NifH). All N_2-fixing bacteria contain a Mo-nitrogenase, and in addition, some species possess one or two non-Mo nitrogenases (Hunter et al., 2009).

1.4.3 Biological phosphorus removal

Phosphorus has been considered a key element leading to eutrophication in aquatic habitats and also a vital element to life (Liang et al., 2010). A wide variety of species of microorganisms, including bacteria, yeasts, fungi, and microalgae, are capable of inorganic polyphosphate (polyP) accumulation above their structural levels (Hiraishi et al., 1991). The microbial synthesis of intracellular polyphosphate in PPB is primarily catalyzed by the enzyme polyphosphate kinase (PPK), which transfers the terminal phosphate of ATP to polyphosphate (Liang et al., 2010). Photosynthetic purple bacteria exhibit a very rich portfolio of membrane lipids including phospholipids not commonly found in bacteria (such as phosphatidylcholine and glycolipids typically found in plant chloroplasts such as sulfoquinovosyl diacylglycerol, betaine and ornithine lipids). These latter lipids lack phosphorus in their structure, presumably allowing PPB to outcompete other organisms in a phosphorus- depleted environment (Hunter et al., 2009).

1.4.4 Heavy metals removal

Similar than microalgae, the mechanisms utilized by PPB for the removal of heavy metals (HMs) are biosorption and bioaccumulation. Some PPB may use more than one mechanisms for the removal of metals. For instance, Panwichian et al., (2011) studied the potential of two PPB strains, *Rhodobium marinum* NW16 and *Rhodobacter sphaeroides* KMS24, to remove HMs from contaminated shrimp pond water. HM were detected in the sediment of shrimp ponds at 0.30 mM Pb^{2+}, 0.89 mM Zn^{2+}, 0.0067 mM Cd^{2+} and 0.54 mM Cu^{2+} (Panwichian et al., 2010). The removal efficiencies of Pb, Zn, Cd and Cu of the two PPB strains tested accounted for 97.29, 91.84, 90.79 and 90.52%, respectively. Likewise, *Rhodobium marinum* NW16 and *Rhodobacter sphaeroides* KMS24 had been used to remove heavy metals and salts from sediments and water collected from contaminated areas to decrease their phytotoxicity (Panwichian et al., 2012).

1.5 Influence of operational and environmental parameters on the activity of algal-bacterial consortia and PPB

1.5.1 pH

The design and operation of biological WWT processes must consider the optimum pH conditions required for microbial growth (Rittmann and McCarty, 2012). The pH of microalgae cultivation broth are typically neutral to alkaline (Rittmann and McCarty, 2012). This can be related to the availability of inorganic carbon in the form of bicarbonate, carbonate and carbon dioxide. When algae grow and extract carbon dioxide from water, pH tends to increase according the following equations [12] and [13]:

$$H_2CO_3 \rightarrow CH_2O + O_2 \quad [12]$$

$$HCO_3^- + H_2O \rightarrow CH_2O + O_2 + OH^- \quad [13]$$

Where, the first reaction illustrates the fact that algal growth results in oxygen production and consumption of carbonic acid, thus causing the pH to rise, according to equation 9. The second reaction indicates that, additionally, if bicarbonates is the carbon source, the hydroxyl ions produced tend to increase the pH of the cultivation broth. On other hand, the bacterial release of CO_2 as a result of organic matter respiration and NH_4^+ nitrification maintain the pH below inhibitory levels. Values of pH between 8.5 and 9.0 have been shown to decrease algal growth, although, some microalgae species can grow at pH values as high as 10-11 (Rittman and Mccarty, 2012). In this regard, pH influences the biosorption capacity of biomass by modifying metal ion solubility and biosorbent total charge, since protons can be absorbed or released (Romera et al., 2007).

On the other hand, most species of bacteria exhibit the optimum range of growth at pH between 6 and 8. In addition, some bacteria, particularly chemolithotrophs that oxidize sulfur or iron for energy production, thrive best under highly acidic conditions. This characteristic enhances their chance for survival, since the end product of their energy metabolism is generally a strong acid (such as sulfuric acid) that prevents competition. PPB are a physiologically versatile group of purple bacteria that can grow well both phototrophically and in darkness. Photosynthesis in purple bacteria occurs at temperatures up to 57 ºC and down to 0 ºC, at pH values as low as 3 or as high as 11, and at salinities up ~32% NaCl (Hunter et al., 2009). Khatipov et al. (1998), who studied the accumulation of poly-ß-hydroxybutyrate by the PPB *Rhodobacter sphaeroides* on various

carbon and nitrogen substrates, observed an increase in pH caused by a decrease in H_2 production and an increase in poly-ß- hydroxybutyrate accumulation on lactate under nitrogen-deprived conditions.

1.5.2 Dissolved oxygen

O_2 is consumed by heterotrophic microorganisms and produced by microalgae during WWT in a algal-bacterial processes (Oswald et al., 1957). According with Rittmann and Mccarty, (2012), 2 mg/L of dissolved oxygen is generally sufficient to maintain an active aerobic organic matter oxidation and nitrification. Hence, Wang et al., (2015) founded that low dissolved oxygen concentration (0.09 - 0.19 mgO_2/L) inhibited nitrite oxidizing bacteria, resulting in stable nitritation. On the other hand, higher dissolved oxygen concentration (< 20 mgO_2/L) combined with an intense irradiance could inhibit microalgae and bacterial activity due to photo-oxidation (Molina et al., 2001). In this context, Molina et al., (2001) determined that microalgae photosynthetic activity was determined as the rate of change in dissolved oxygen concentration. However, the dissolved oxygen concentration in the cultivation of microalgae-based WWT processes remains typically low as result of the active consumption by bacterial and heterotrophic microalgae.

On the other hand, many purple sulfur bacteria and purple non-sulfur bacteria are extremely sensitive to oxygen (Talaiekhozani and Rezania, 2017). Thus, oxygen partial pressure is a major factor regulating the formation of the photosynthetic apparatus and the cell differentiation of most facultative purple phototrophic bacteria capable of respiratory and photosynthetic modes of energy transduction. Two species forming the photosynthetic apparatus under both aerobic and anaerobic conditions are exceptions to this generalization: *Rhodopseudomonas sulfidophil* and Rhodospirillum *centenum* (Yurkov and Beatty, 1998). Thus, the competitive success of purple bacteria in nature requires light and anoxic conditions (Hunter et al., 2009). However, Meng et al., (2017), who worked with *Rhodopseudomonas* during synthetic wastewater treatment in batch tests, reported the highest COD (93%) and NH_3-N removal (83%) under DO of 4–8 mg/L.

1.5.3 Temperature

Temperature plays an important role in algal-bacterial processes devoted to WWT (Mobin and Alam, 2014). Temperatures influence the rate of all biochemical reactions involved in bacterial and microalgal growth, with higher rates observed when temperature increases. However, at very high temperatures, key enzymes are denaturalized and the organism may not survive. Despite some extremophile microalgae can grow at 0 °C and others at 90 °C, the optimal range for microalgae activity lies in 20-30 ºC (Rittmann and McCarty, 2012)(Singh and Singh, 2015). Talbot et al., (1991) compared microalgae growth at different temperature and observed no significant difference between *P. bohneri* and *A. falcatus* at 25, 30 and 35 °C in batch test using an artificial medium. Ho et al., (2012) reported that 28 °C improved CO_2 fixation of indigenous *Scenedesmus obliquus* in a tubular glass photobioreactor of 1L. Studies carried out under outdoors conditions in two identical HRAPs with temperatures ranging between 11 °C in January and 25 °C in July reported Chlorophyll ***a*** concentrations higher (3.0 to 4.0 mg/L) during the spring and summer than during autumn and winter (1.0 to 2.0 mg/L) (García et al., 2000).

PPB are able to grow at temperatures ranging from 0 to 57 ºC (Hunter et al., 2009). The temperatures ranges prevailing during wastewater treatment using purple phototrophic bacteria are similar to those recorded when using algal-bacterial systems. For instance, Kantachote et al., (2005) reported 30 °C as optimal temperature for cell growth during the treating rubber sheet wastewater enrichment with PPB (*Rhodopseudomonas blastica*). Likewise Madukasi et al., (2010) used a temperature between 20 and 30 °C during the treatment of pharmaceutical wastewater by a wild PPB strain named Z08 and identified as *Rhodobacter-sphaeroides* isolated from soil.

1.5.4 Photosynthetically active radiation

Light in the range 400 to 700 nm of the electromagnetic spectrum provides the energy for photosynthesis and plays an central role in the performance of algal-bacterial processes (Figure 9) (MacDonald, 2003). The solar radiation available for photosynthesis inside the cultivation broth of the microalgae based photobioreactor controls the rate of carbon fixation and therefore biomass productivity and WWT performance (Frouin and Pinker, 1995) According with Ogbonna and Tanaka (2000), light is the most critical factor in

photobioreactor and very often the factor limiting algal photosynthesis. Cheloni et al., (2014) showed the important role of light intensity and spectral composition on Cu uptake and the effects on the green alga *Chlamydomonas reinhardtii*. High irradiations increased cellular Cu concentrations, but mitigated the Cu-induced decrease in chlorophyll fluorescence, oxidative stress and lipid peroxidation at high Cu concentrations, indicating that Cu and high irradiations interact in an antagonistic manner.

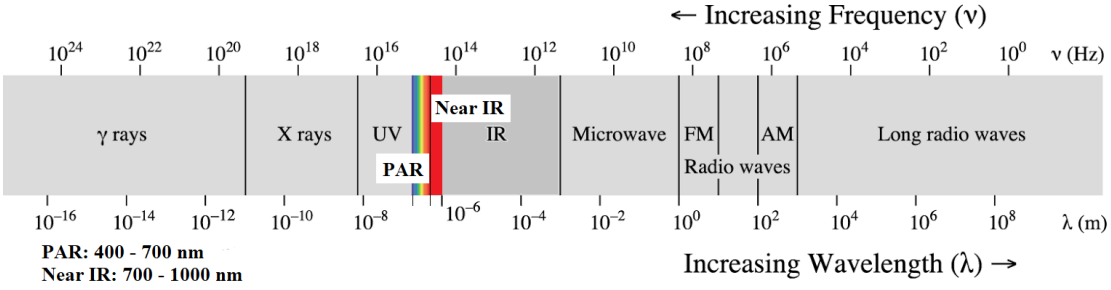


Figure 9. The electromagnetic spectrum (source: http://schoolbag.info/physics/physics_math)

During photosynthesis (Figure 10), inorganic compounds and light energy are converted into organic matter by photoautotrophs (Richmond, 2004). Microorganisms obtain their energy for growth and maintenance from Redox reactions triggered by the photon captured from solar light. Microalgae use oxidation-reduction reactions to convert the light energy into ATP and NAD (Rittman and Mccarty, 2012).

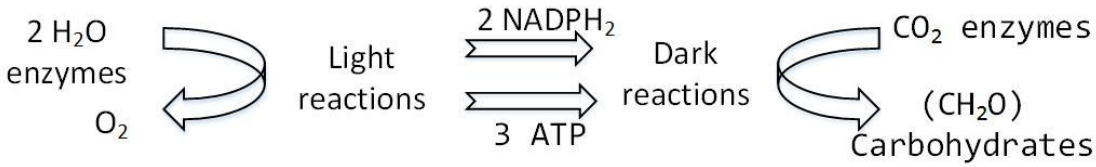


Figure 10. Products of the light and dark reactions of photosynthesis (adapted from Hall & Rao, 1999). Source (Richmond, 2004).

According with Cogdell and Thornber, (1980) PPB can absorb radiation in the wavelength range between 800 and 900 nm due to the ability of the harvesting complexes present in the bacteriochlorophyll (Figure 9). All PPBs have in-vivo bacteriochlorophyll and carotenoids that carry out photosynthesis without oxygen production and their photosynthesis rates depends not only on the infrared light intensity but on the supply of external electron donors such as sulfide, molecular hydrogen or organic substances (Kantachote et al., 2005; Madukasi et al., 2011). Bertling et al., (2006) demonstrated the feasibility of PPB growth supported by a Laser Diode (in infrared spectrum) irradiating

at 30 W/m^2. Likewise, Hülsen et al., (2013) used infrared selected PPB as a platform for the biological removal of carbon, nitrogen and phosphorous under anaerobic conditions with removal efficiencies of COD of 63 ± 5%, NH_4^+-N of 99.6% and PO_4^{3-}-P of 88% from primary settled domestic wastewater in 24 h.

1.5.5 Evaporation rates

Evaporation is an important parameter during the wastewater treatment in open photobioreactors based on its pollutant concentration effect. In this context, Guieysse et al., (2013) have highlighted that evaporation has a critical impact on the economics and sustainability of algae mass cultivation in open systems. The high evaporation rates in open photobioreactors treating fish processing wastewater exerted a negative impact on the biomass growth (Riaño et al., 2012).The main factors impacting on water evaporation in photobioreactors are solar radiation, temperature, relative humidity, vapour pressure deficit, atmospheric pressure and wind (Kumar et al., 2016). Posadas et al., (2015) reported evaporation losses ranging from 4.4 ± 1.4 to 7.3 ± 0.2 L $m^{-2}.d^{-1}$ as a result of the operational conditions in a 180 L HRAP. The main cause of the high water evaporation losses was the high turbulence as a consequence of its pilot scale design, where the paddlewheel engine was oversized.

1.5.6 Hydraulic retention times

HRAP are typically operated at 2-6 days HRT and similar values have been reported in enclosed photobioreactors (Muñoz and Guieysse, 2006). Aguirre et al., (2011) set 40 days of HRT during the treatment of PWW with a COD of 3000 mgO_2/L using and HRAP of 400L. However, de Godos et al., (2009a) operated with 10 days of HRT in a 464L HRAP treating 20 and 10 folds diluted swine manure.

1.5.7 C/N/P ratio

The most important limitation of biological processes during urban and livestock wastewater treatment is the low C:N:P ratio of these types of wastewaters (Arbib et al., 2013), which ideally should match with the typical biomass composition represented by the Redfield ratio C:N:P: 106/16/1 (Richmond, 2004). Most research treating domestic, industrial, agroindustrial and livestock effluents in microalgae-based processes were operated under carbon limitation (Arbib et al., 2013).

1.6 Photobioreactors

Photobioreactors have emerged as an alternative platform to treat wastewater using algal-bacterial consortia or phototrophic purple bacteria. Photobioreactors used for wastewater treatment have similar design and operational criteria than conventional photobioreactor used for mass cultivation. For instance, a high surface/volume ratio to maximize light utilization efficiency (and therefore oxygen production), adequate mixing and degassing, good scalability, low hydrodynamic stress on the algal-bacterial flocs, control over the environmental conditions and low construction and operation costs rank among the top characteristics desired in photobioreactors devoted to WWT (Muñoz and Guieysse, 2006; Richmond, 2004). However, the most import issue to design a photobioreactor are related to light supply, which represents the most costly parameter to optimize (Adessi and De Philippis, 2014).

1.6.1 Closed photobioreactors

Closed photobioreactors are characterized by the regulation and control of nearly all the environmental parameters as well as by the following fundamental benefits: a reduced contamination risk, no CO_2 losses, reproducible cultivation conditions, controllable hydrodynamics and temperature, and a flexible technical design (Pulz, 2001). Closed photobioreactors support higher photosynthetic efficiencies as a result of their higher illuminated area to volume ratio and a better control on operational parameters than open systems (Muñoz and Guieysse, 2006). The main disadvantages of these photobioreactors derive from the high operational cost to maintain the optimal temperature to avoid a significant productivity drop (Béchet et al., 2010). Closed photobioreactors are currently used for microalgae mass cultivation in the following configurations: tubular (Figure 11) and flat-plate (Figure 12). Tubular photobioreactors (vertical, horizontal and helical) are the only type of closed systems used at large scale. The optimum diameter of the tubes in TPBRs range from 3 to 12 cm since smaller diameters could cause biomass clogging, while the recommended linear velocity in the tubes ranges from 20 to 50 $cm \cdot s^{-1}$, which is normally controlled by the use of airlift systems or centrifugal pumps (Christenson and Sims, 2011) (González-Fernández and Muñoz, 2017).

Figure 11. Tubular photobioreactor configurations (Credit: Image: Photobioreactor PBR 4000 G IGV Biotech)

Figure 12. Vertical-plate photobioreactor (Credit: Image: Photobioreactor PBR 500 P IGV Biotech)

Flat-plate photobioreactors provide an optimized light distribution. In addition, their simpler construction compared to tubular reactors allows the use of less expensive plastic materials. Flat plate systems are characterized by an open gas transfer area, thus reducing the need for a dedicated degassing unit (Dasgupta et al., 2010)(Carvalho and Meireles, 2006).

1.6.2 Open photobioreactors

High rate algal ponds, also named raceway ponds, are most common open photobioreactors, whose design and operational parameters depend on the influent characteristics and environmental conditions such as temperature, irradiance and evaporation losses (Alcántara et al., 2015c; Muñoz and Guieysse, 2006). HRAPs have been used since 1950´s and are typically constructed based on a design criterion of 11 m^2 per population equivalent (Oswald et al., 1957)(European Union Commission (2001). HRAPs are 2-3 m wide and 0.1- 0.3 m depth shallow open ponds built in a raceway configuration, lined with PVC, clay or asphalt to avoid infiltration. The surface of these photobioreactors range from 1000 to 10000 m^2 in large-scale applications (Muñoz and Guieysse, 2006). Three open photobioreactor configurations have been up-scaled: raceways ponds, circular ponds and thin layer systems. The latter exhibit limitations such as the sedimentation of cells at points of lower turbulence, strong evaporative losses, high rates of CO_2 desorption and considerable requirement of energy for continuously pumping the culture to the head of the inclined surface (Richmond, 2004). Figures 13 and 14 depicts the most common open photobiorector configurations used for wastewater treatment.

Figure 6. HRAP with a thick dividing section. In this picture, the water flows in a clockwise direction powered by two paddle wheels. (Credit: SARDI: South Australian Research and Development Institute)

Figure 14. Circular raceway for microalgae cultivation of 5 m^2. (Source: Wang et al., 2018)

The main disadvantage of these photobioreactor configuration is the large treatment area needed to complete phosphorus removal compared to the area needed to remove of carbon and nitrogen (Alcántara et al., 2015c). Under optimal conditions, HRAPs can treat up to 35 g·BOD·m^{-2}·d^{-1} (175 g·BOD·$m^{-3}d^{-1}$ in a 0.2 m deep pond) compared to 5–10 g·BOD·m^{-2}·d^{-1} (5-10 g BOD·m^{-3} d^{-1} in a 1 m deep pond) in waste stabilization ponds (Muñoz and Guieysse, 2006; Racault and Boutin, 2005).

1.7 Biomass valorization

Biomass from domestic and agroindustrial wastewater treatment can be used as a feedstock to produce biofertilizers based on its high carbon and nutrients concentration. Indeed, Cabanelas et al., (2013) reported contents of 40-60%, 4-7% and 0.1-1% of carbon, nitrogen and phosphorus, respectively in biomass grown in a photobioreactor inoculated with *Chlorella vulgaris* and supplemented with CO_2 during the treatment of effluent from primary settler. In this context, limitation in nitrogen or phosphorus could result in an increase in the carbon content of the harvested biomass (mainly composed of proteins (40%-60%), lipids (5%-60%) and carbohydrates (8%-30%)) (Markou et al., 2014)(Tijani et al., 2015). Algae-based fuels or biofuels offer many advantages compared to conventional fossil fuels (Balat, 2011). Production of bioethanol from algal biomass is one alternative to reduce the demand of fuels from petroleum and its associated environmental pollution (Balat, 2011). Bioethanol has received in the past decades an increasing attention worldwide. However, the cost of bioethanol production is still higher

than that of fossil fuels (Sarkar et al., 2012). Biodiesel production is another alternative algae-based fuel which can be used as substitute or additive of conventional diesel (Vivek and Gupta, 2004). However, the lipid content of biomass grown in WWT plants is usually low. In this context, Álvarez-Díaz et al., (2015) increased the lipid (from 35.8% to 49%) content of ω-3 eicosapentaenoic acid in *Scenedesmus obliquus* treating domestic wastewater, which opened the door to develop wastewater-to-biodiesel strategies during microalgae-based WWT. Biohydrogen is another biofuel produced from or by microalgae (Chandrasekhar et al., 2015). Biological production of hydrogen (Biohydrogen) using microalgae as a feedstock or biocatalyst include direct biophotolysis, indirect biophotolysis, photo-fermentations, dark-fermentation, microbial electrolysis cell, multi-stage integrated process and water-gas shift (Levin et al., 2004) (Holladay et al., 2009). Finally, microalgae can be converted into biogas via anaerobic digestion (Converti et al., 2009). In this context, the biogas produced from algal biomass contain a high-energy value and the energy recovery is comparable to that of the extraction from cell lipids. Due to its simplicity and extensive experience in design and operation of anaerobic digesters, biogas is becoming the most popular biofuel produced from microalgae (Wayne Chew et al., 2017).

1.8 References

1. Abe, K., Waki, M., Suzuki, K., Kasuya, M., Suzuki, R., Itahashi, S., Banzai, K., 2012. Estimation of Zn and Cu unit output loads from animal husbandry facilities. Water Science and Technology 66, 653–658. doi:10.2166/wst.2012.224

2. Adessi, A., De Philippis, R., 2014. Photobioreactor design and illumination systems for H2 production with anoxygenic photosynthetic bacteria: A review. International Journal of Hydrogen Energy 39, 3127–3141. doi:10.1016/j.ijhydene.2013.12.084

3. Aguirre, P., Álvarez, E., Ferrer, I., García, J., 2011. Treatment of piggery wastewater in experimental high rate algal ponds. Rev Latinoam Biotecnol Amb Algal 2, 57–6657.

4. Ahlf, W., 1988. Recovery of metals from acid waste water by Cyanidium caldarium. Applied Microbiology and Biotechnology 28, 512–513. doi:10.1007/BF00268224

5. Ahuja, P., Gupta, R., Saxena, R.K., 1999. Zn2+ biosorption by Oscillatoria anguistissima. Process Biochemistry 34, 77–85. doi:10.1016/S0032-9592(98)00072-7

6. Alcántara, C., Domínguez, J.M., García, D., Blanco, S., Pérez, R., García-Encina, P.A., Muñoz, R., 2015a. Evaluation of wastewater treatment in a novel anoxic-aerobic algal-bacterial photobioreactor with biomass recycling through carbon and nitrogen mass balances. Bioresource Technology 191, 173–186. doi:10.1016/j.biortech.2015.04.125

7. Alcántara, C., García-Encina, P.A., Muñoz, R., 2015b. Evaluation of the simultaneous biogas upgrading and treatment of centrates in a high-rate algal pond through C, N and P mass balances. Water Science and Technology 72, 150–157. doi:10.2166/wst.2015.198

8. Alcántara, C., Posadas, E., Guieysse, B., Muñoz, R., 2015c. Microalgae-based Wastewater Treatment. Handbook of Marine Microalgae: Biotechnology Advances 439–455. doi:10.1016/B978-0-12-800776-1.00029-7

9. Álvarez-Díaz, P.D., Ruiz, J., Arbib, Z., Barragán, J., Garrido-Pérez, M.C., Perales, J. a., 2015. Wastewater treatment and biodiesel production by Scenedesmus obliquus in a two-stage cultivation process. Bioresource Technology 181, 90–96. doi:10.1016/j.biortech.2015.01.018

10. Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C., Barragan, J., Perales, J.A., 2013. Effect of pH control by means of flue gas addition on three different photo-bioreactors treating urban wastewater in long-term operation. Ecological Engineering 57, 226–235. doi:10.1016/j.ecoleng.2013.04.040

11. Azad, S.A., Vikineswary, S., Ramachandran, K.B., Chong, V.C., 2001. Growth and production of biomass of Rhodovulum sulfidophilum in sardine processing wastewater. Letters in Applied Microbiology 33, 264–268. doi:10.1046/j.1472-765X.2001.00993.x

12. Balat, M., 2011. Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. Energy Conversion and Management 52, 858–875. doi:10.1016/j.enconman.2010.08.013

13. Basak, N., Das, D., 2007. The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: The present state of the art. World Journal of Microbiology and Biotechnology 23, 31–42. doi:10.1007/s11274-006-9190-9

14. Béchet, Q., Shilton, A., Fringer, O.B., Munoz, R., Guieysse, B., 2010. Mechanistic modeling of broth temperature in outdoor photobioreactors. Environmental Science and Technology 44, 2197–2203. doi:10.1021/es903214u

15. Bertling, K., Hurse, T.J., Kappler, U., Rakic, A.D., Sciences, M., 2006. Lasers — An Effective Artificial Source of Radiation for the Cultivation of Anoxygenic Photosynthetic Bacteria. Biotechnology and Bioengineering 94, 337–345. doi:10.1002/bit

16. Cabanelas, I.T.D., Ruiz, J., Arbib, Z., Chinalia, F.A., Garrido-Pérez, C., Rogalla, F., Nascimento, I.A., Perales, J.A., 2013. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresource Technology 131, 429–436. doi:10.1016/j.biortech.2012.12.152

17. Cai, T., Park, S.Y., Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renewable and Sustainable Energy Reviews. doi:10.1016/j.rser.2012.11.030

18. Carvalho, A.P., Meireles, L.A., 2006. Microalgae reactors: A review of enclosed systems and performances. Biotechnology progress 3, 1490–1506.

19. Chandrasekhar, K., Lee, Y.-J., Lee, D.-W., 2015. Biohydrogen Production: Strategies to Improve Process Efficiency through Microbial Routes. Int. J. Mol. Sci. Int. J. Mol. Sci 16, 8266–8293. doi:10.3390/ijms16048266

20. Chaumont, D., 1993. Biotechnology of algal biomass production: a review of systems for outdoor mass culture. Journal of Applied Phycology 5, 593–604. doi:10.1007/BF02184638

21. Cheah, W.Y., Ling, T.C., Show, P.L., Juan, J.C., Chang, J.S., Lee, D.J., 2016. Cultivation in wastewaters for energy: A microalgae platform. Applied Energy 179, 609–625. doi:10.1016/j.apenergy.2016.07.015

22. Cheah, W.Y., Show, P.L., Chang, J.S., Ling, T.C., Juan, J.C., 2015. Biosequestration of atmospheric CO2 and flue gas-containing CO2 by microalgae. Bioresource Technology 184, 190–201. doi:10.1016/j.biortech.2014.11.026

23. Cheloni, G., Cosio, C., Slaveykova, V.I., 2014. Antagonistic and synergistic effects of light irradiation on the effects of copper on Chlamydomonas reinhardtii. Aquatic toxicology (Amsterdam, Netherlands) 155C, 275–282. doi:10.1016/j.aquatox.2014.07.010

24. Cho, S., Lee, N., Park, S., Yu, J., Luong, T.T., Oh, Y.-K., Lee, T., 2013. Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources. Bioresource Technology 131, 515–520. doi:10.1016/j.biortech.2012.12.176

25. Choi, H.J., Lee, J.M., Lee, S.M., 2013. A novel optical panel photobioreactor for cultivation of microalgae. Water Science and Technology 67, 2543–2548. doi:10.2166/wst.2013.128

26. Chojnacka, K., Chojnacki, A., Górecka, H., 2005. Biosorption of Cr3+, Cd2+and Cu2+ions by blue-green algae Spirulina sp.: Kinetics, equilibrium and the mechanism of the process. Chemosphere 59, 75–84. doi:10.1016/j.chemosphere.2004.10.005

27. Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnology advances 29, 686–702. doi:10.1016/j.biotechadv.2011.05.015

28. Cogdell, R.J., Thornber, J.P., 1980. Light-harvesting pigment-protein complexes of purple

photosynthetic bacteria. FEBS Letters 122, 1–8. doi:10.1016/0014-5793(80)80388-7

29. Converti, A., Oliveira, R.P.S., Torres, B.R., Lodi, A., Zilli, M., 2009. Biogas production and valorization by means of a two-step biological process. Bioresource technology 100, 5771–6. doi:10.1016/j.biortech.2009.05.072

30. Cuellar-Bermudez, S.P., Romero-Ogawa, M.A., Vannela, R., Lai, Y.S., Rittmann, B.E., Parra-Saldivar, R., 2015. Effects of light intensity and carbon dioxide on lipids and fatty acids produced by Synechocystis sp. PCC6803 during continuous flow. Algal Research 12, 10–16. doi:10.1016/j.algal.2015.07.018

31. Dasgupta, C.N., Jose Gilbert, J., Lindblad, P., Heidorn, T., Borgvang, S.A., Skjanes, K., Das, D., 2010. Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. International Journal of Hydrogen Energy 35, 10218–10238. doi:10.1016/j.ijhydene.2010.06.029

32. Dassey, A.J., Theegala, C.S., 2013. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresource Technology 128, 241–245. doi:10.1016/j.biortech.2012.10.061

33. de Godos, I., Blanco, S., García-Encina, P.A., Becares, E., Muñoz, R., 2009a. Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates. Bioresource Technology 100, 4332–4339. doi:10.1016/j.biortech.2009.04.016

34. de Godos, I., González, C., Becares, E., García-Encina, P.A., Muñoz, R., 2009b. Simultaneous nutrients and carbon removal during preateated swine slurry degradation in a tubular biofilm photobioreactor. Applied Microbiology and Biotechnology 82, 187–194. doi:10.1007/s00253-008-1825-3

35. de Godos, I., Vargas, V.A., Blanco, S., González, M.C.G., Soto, R., García-Encina, P.A., Becares, E., Muñoz, R., 2010. A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. Bioresource Technology 101, 5150–5158. doi:10.1016/j.biortech.2010.02.010

36. de Godos, I., Vargas, V.A., Guzmán, H.O., Soto, R., García, B., García, P.A., Muñoz, R., 2014. Assessing carbon and nitrogen removal in a novel anoxic-aerobic cyanobacterial-bacterial photobioreactor configuration with enhanced biomass sedimentation. Water Research 61, 77–85. doi:10.1016/j.watres.2014.04.050

37. de la Noüe, J., Laliberté, G., Proulx, D., 1992. Algae and waste water. Journal of Applied Phycology 4, 247–254. doi:10.1007/BF02161210

38. De la Torre, A.I., Jiménez, J.A., Carballo, M., Fernandez, C., Roset, J., Muñoz, M.J., 2000. Ecotoxicological evaluation of pig slurry. Chemosphere 41, 1629–1635. doi:10.1016/S0045-6535(00)00038-2

39. de Lima, L.K.F., Ponsano, E.H.G., Pinto, M.F., 2011. Cultivation of Rubrivivax gelatinosus in fish industry effluent for depollution and biomass production. World Journal of Microbiology and Biotechnology 27, 2553–2558. doi:10.1007/s11274-011-0725-3

40. Diameter, S., Sewers, G., 2000. Decentralized Systems Technology Fact Sheet. Environmental Protection 1–4.

41. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E., 2006. The

Denitrifying Prokaryotes, in: Dworkin, M., Falkow, S. (Eds.), The Prokaryotes. doi:10.1007/978-3-642-38922-1

42. European Parliament, C. of the E., 2003. Directive 2003/55/EC of the European Parliament and of the Council of 26 June 2003 concerning common rules for the internal market in natural gas and repealing Directive 98/30/EC. Official Journal of the European Union 57–78.

43. European Union Commission, 2001. Extensive Wastewater Treatment Processes Adapted to Small and Medium Sized communitites.

44. Evans, L., Hennige, S.J., Willoughby, N., Adeloye, A.J., Skroblin, M., Gutierrez, T., 2017. Effect of organic carbon enrichment on the treatment efficiency of primary settled wastewater by Chlorella vulgaris. Algal Research 24, 368–377. doi:10.1016/j.algal.2017.04.011

45. Frouin, R., Pinker, R.T., 1995. Estimating Photosynthetically Active Radiation (PAR) at the earth's surface from satellite observations. Remote Sensing of Environment 51, 98–107. doi:10.1016/0034-4257(94)00068-X

46. García Ferrero, J.L., 2013. Tecnología de la gestión y tratamiento de purines. Aplicación en la agricultura y otros usos [WWW Document]. URL http://racve.es/publicaciones/tecnologia-de-la-gestion-y-tratamiento-de-purines-aplicacion-en-la-agricultura-y-otros-usos/ (accessed 6.7.15).

47. García, J., Mujeriego, R., Hernández-Mariné, M., 2000. Biogas Upgrading via Hydrogenotrophic Methanogenesis in Two-Stage Continuous Stirred Tank Reactors at Mesophilic and Thermophilic Conditions. Applied Phycology 12, 331–339. doi:10.1023/a:1008146421368

48. Goldman, J.C., Porcella, D.B., Joe Middlebrooks, E., Toerien, D.F., 1972. The effect of carbon on algal growth-Its relationship to eutrophication. Water Research 6, 637–679. doi:10.1016/0043-1354(72)90182-0

49. González-Fernández, C., Muñoz, R., 2017. Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-products, Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-products. Elsevier Ltd.

50. Guieysse, B., Béchet, Q., Shilton, A., 2013. Variability and uncertainty in water demand and water footprint assessments of fresh algae cultivation based on case studies from five climatic regions. Bioresource Technology 128, 317–323. doi:10.1016/j.biortech.2012.10.096

51. Gupta, P., Diwan, B., 2017. Bacterial Exopolysaccharide mediated heavy metal removal: A Review on biosynthesis, mechanism and remediation strategies. Biotechnology Reports 13, 58–71. doi:10.1016/j.btre.2016.12.006

52. Gutiérrez, R., Passos, F., Ferrer, I., Uggetti, E., García, J., 2015. Harvesting microalgae from wastewater treatment systems with natural flocculants: Effect on biomass settling and biogas production. Algal Research 9, 204–211. doi:10.1016/j.algal.2015.03.010

53. Hao, R., Li, S., Li, J., Meng, C., 2013. Denitrification of simulated municipal wastewater treatment plant effluent using a three-dimensional biofilm-electrode reactor: operating performance and bacterial community. Bioresource technology 143, 178–86. doi:10.1016/j.biortech.2013.06.001

54. Hassan, M.A., Shirai, Y., Kusubayashi, N., Karim, M.I.A., Nakanishi, K., Hashimoto, K., 1997. The production of polyhydroxyalkanoate from anaerobically treated palm oil mill effluent by Rhodobacter

sphaeroides. Journal of Fermentation and Bioengineering 83, 485–488. doi:10.1016/S0922-338X(97)83007-3

55. Heubeck, S., Craggs, R.J., Shilton, A., 2007. Influence of CO2 scrubbing from biogas on the treatment performance of a high rate algal pond. Water Science and Technology 55, 193–200. doi:10.2166/wst.2007.358

56. Hiraishi, A., Yanase, A., Kitamura, H., 1991. Polyphosphate Accumulation by Rhodobacter Grown under Different Environmental Conditions with Special Emphasis on the Effect of External Phosphate Concentrations 6, 25–32.

57. Ho, S.-H., Lu, W.-B., Chang, J.-S., 2012. Photobioreactor strategies for improving the CO2 fixation efficiency of indigenous Scenedesmus obliquus CNW-N: Statistical optimization of CO2 feeding, illumination, and operation mode. Bioresource Technology 105, 106–113. doi:10.1016/j.biortech.2011.11.091

58. Holladay, J.D., Hu, J., King, D.L., Wang, Y., 2009. An overview of hydrogen production technologies. Catalysis Today 139, 244–260. doi:10.1016/j.cattod.2008.08.039

59. Hügler, M., Sievert, S.M., 2011. Beyond the Calvin Cycle: Autotrophic Carbon Fixation in the Ocean. Annual Review of Marine Science 3, 261–289. doi:10.1146/annurev-marine-120709-142712

60. Hülsen, T., Barry, E.M., Lu, Y., Puyol, D., Batstone, D.J., 2016a. Low temperature treatment of domestic wastewater by purple phototrophic bacteria: Performance, activity, and community. Water Research 100, 537–545. doi:10.1016/j.watres.2016.05.054

61. Hülsen, T., Barry, E.M., Lu, Y., Puyol, D., Keller, J., Batstone, D.J., 2016b. Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor. Water Research 100, 486–495. doi:10.1016/j.watres.2016.04.061

62. Hülsen, T., Batstone, D.J., Keller, J., 2014. Phototrophic bacteria for nutrient recovery from domestic wastewater. Water Research 50, 18–26. doi:10.1016/j.watres.2013.10.051

63. Hunter, C.N., Daldal, F., Thurnauer, M.C., Beatty, J.T., 2009. The Purple Phototrophic Bacteria. doi:10.1007/978-1-4020-8815-5

64. Ihp, 2008. The impact of global change on water resources 21.

65. Javanbakht, V., Alavi, S.A., Zilouei, H., 2014. Mechanisms of heavy metal removal using microorganisms as biosorbent. Water Science and Technology 69, 1775–1787. doi:10.2166/wst.2013.718

66. Kantachote, D., Torpee, S., Umsakul, K., 2005. The potential use of anoxygenic phototrophic bacteria for treating latex rubber sheet wastewater. Electronic Journal of Biotechnology 8, 314–323. doi:10.2225/vol8-issue3-fulltext-8

67. Khatipov, E., Miyake, M., Miyake, J., Asada, Y., 1998. Accumulation of poly-ß-hydroxybutyrate by Rhodobacter sphaeroides on various carbon and nitrogen substrates. FEMS Microbiology Letters 162, 39–45. doi:10.1111/j.1574-6968.1998.tb12976.x

68. Kumar, P., Panwar, R., Kumar, D., 2016. Evaporation estimation from climatic factors. Mausam 67, 897–902.

69. Kuo, C.M., Chen, T.Y., Lin, T.H., Kao, C.Y., Lai, J.T., Chang, J.S., Lin, C.S., 2015. Cultivation of Chlorella sp. GD using piggery wastewater for biomass and lipid production. Bioresource Technology 194, 326–333. doi:10.1016/j.biortech.2015.07.026

70. Lau, P.S., Tam, N.F.Y., Wong, Y.S., 1995. Effect of algal density on nutrient removal from primary settled wastewater. Environmental Pollution 89, 59–66. doi:10.1016/0269-7491(94)00044-E

71. Lavoie, a, de la Noüe, J., 1985. Hyperconcentrated cultures of Scenedesmus obliquus. Water Research 19, 1437–1442. doi:10.1016/0043-1354(85)90311-2

72. Lee, Y.-S., Han, G.-B., 2016. Complete reduction of highly concentrated contaminants in piggery waste by a novel process scheme with an algal-bacterial symbiotic photobioreactor. Journal of Environmental Management 177, 202–212. doi:10.1016/j.jenvman.2016.04.025

73. Levin, D.B., Pitt, L., Love, M., 2004. Biohydrogen production: Prospects and limitations to practical application. International Journal of Hydrogen Energy 29, 173–185. doi:10.1016/S0360-3199(03)00094-6

74. Li, T., Lin, G., Podola, B., Melkonian, M., 2015. Continuous removal of zinc from wastewater and mine dump leachate by a microalgal biofilm PSBR. Journal of Hazardous Materials 297, 112–118. doi:10.1016/j.jhazmat.2015.04.080

75. Liang, C.M., Hung, C.H., Hsu, S.C., Yeh, I.C., 2010. Purple nonsulfur bacteria diversity in activated sludge and its potential phosphorus-accumulating ability under different cultivation conditions. Applied Microbiology and Biotechnology 86, 709–719. doi:10.1007/s00253-009-2348-2

76. Liu, J., Wu, Y., Wu, C., Muylaert, K., Vyverman, W., Han-Qing, Y., Muñoz, R., Rittmann, B., 2017. Advanced nutrient removal from surface water by a consortium of attached microalgae and bacteria: A review. Bioresource Technology 241, 1127–1137. doi:10.1016/j.biortech.2017.06.054

77. Luongo, V., Ghimire, A., Frunzo, L., Fabbricino, M., d'Antonio, G., Pirozzi, F., Esposito, G., 2017. Photofermentative production of hydrogen and poly-B-hydroxybutyrate from dark fermentation products. Bioresource Technology 228, 171–175. doi:10.1016/j.biortech.2016.12.079

78. MacDonald, G.M., 2003. Space , Time , and Life. John Wiley & Sons, Inc, New York, USA.

79. Madukasi, E.I., Chunhua, H., Zhang, G., 2011. Isolation and application of a wild strain photosynthetic bacterium to environmental waste management. Int. J. Environ. Sci. Tech 8, 513–522.

80. Madukasi, E.I., Dai, X., He, C., Zhou, J., 2010. Potentials of phototrophic bacteria in treating pharmaceutical wastewater. Int. J Environ. Sci. Tech 7, 165–174.

81. Marín, D., Posadas, E., Cano, P., Pérez, V., Lebrero, R., Muñoz, R., 2018. Influence of the seasonal variation of environmental conditions on biogas upgrading in an outdoors pilot scale high rate algal pond. Bioresource Technology 255, 354–358. doi:10.1016/j.biortech.2018.01.136

82. Markou, G., Vandamme, D., Muylaert, K., 2014. Microalgal and cyanobacterial cultivation: The supply of nutrients. Water research 65, 186–202. doi:10.1016/j.watres.2014.07.025

83. Meng, F., Yang, A., Zhang, G., Wang, H., 2017. Effects of dissolved oxygen concentration on photosynthetic bacteria wastewater treatment: Pollutants removal, cell growth and pigments production. Bioresource Technology 241, 993–997. doi:10.1016/j.biortech.2017.05.183

84. Metcalf, E., Eddy, H., 2003. Wastewater engineering: treatment and reuse. Tata McGraw-Hill Publishing Company Limited, 4th edition. New Delhi, India. doi:10.1016/0309-1708(80)90067-6

85. Mobin, S., Alam, F., 2014. Biofuel production from algae utilizing wastewater. Proceedings of the 19th Australasian Fluid Mechanics Conference, AFMC 2014.

86. Mohamed, R.M., 2015. Biosorption of zinc and cadmium by Klebsiella pneumonia KM609983

isolated from Sohag, Egypt. Global Advanced Research Journal of Microbiology 4, 2315–5116.

87. Molina, E., Fernández, J., Acién, F.G., Chisti, Y., 2001. Tubular photobioreactor design for algal cultures. Journal of Biotechnology 92, 113–131. doi:10.1016/S0168-1656(01)00353-4

88. Muñoz, A.J., Ruiz, E., Abriouel, H., Gálvez, A., Ezzouhri, L., Lairini, K., Espínola, F., 2012. Heavy metal tolerance of microorganisms isolated from wastewaters: Identification and evaluation of its potential for biosorption. Chemical Engineering Journal 210, 325–332. doi:10.1016/j.cej.2012.09.007

89. Muñoz, R., Alvarez, M.T., Muñoz, A., Terrazas, E., Guieysse, B., Mattiasson, B., 2006. Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. Chemosphere 63, 903–11. doi:10.1016/j.chemosphere.2005.09.062

90. Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: A review. Water Research 40, 2799–2815. doi:10.1016/j.watres.2006.06.011

91. Myung, K.K., Choi, K.M., Yin, C.R., Lee, K.Y., Im, W.T., Ju, H.L., Lee, S.T., 2004. Odorous swine wastewater treatment by purple non-sulfur bacteria, Rhodopseudomonas palustris, isolated from eutrophicated ponds. Biotechnology Letters 26, 819–822. doi:10.1023/B:BILE.0000025884.50198.67

92. Nations, U., 2017. Wastewater should be recognized as a valuable resource, UN says on World Water Day [WWW Document]. URL http://www.un.org/apps/news/story.asp?NewsID=56397#.WSK5oWjyiUn (accessed 5.22.17).

93. Ogbonna, J.C., Tanaka, H., 2000. Light requirement and photosynthetic cell cultivation – Development of processes for efficient light utilization in photobioreactors. Journal of Applied Phycology 12, 207–218. doi:10.1023/A:1008194627239

94. Olguín, E.J., 2003. Phycoremediation: Key issues for cost-effective nutrient removal processes. Biotechnology Advances 22, 81–91. doi:10.1016/S0734-9750(03)00130-7

95. Olguín, E.J., Mendoza, A., González-Portela, R.E., Novelo, E., 2013. Population dynamics in mixed cultures of Neochloris oleoabundans and native microalgae from water of a polluted river and isolation of a diatom consortium for the production of lipid rich biomass. New Biotechnology 30, 705–715. doi:10.1016/j.nbt.2013.03.003

96. Omar, H.H., 2002. Adsorption of zinc ions by Scenedesmus obliquus and S. quadricauda and its effect on growth and metabolism. Biologia Plantarum. doi:10.1023/A:1015157009150

97. Oswald, W.J., Gotaas, H.B., Golueke, C.G., Kellen, W.R., Gloyna, E.F., Hermann, E.R., 1957. Algae in Waste Treatment [with Discussion]. Water Environment Federation 29, 437–457. doi:10.2307/25033322

98. Overmann, J., García-Pichel, F., 2013. The phototrophic way of life. The Prokaryotes: Prokaryotic Communities and Ecophysiology 9783642301, 203–257. doi:10.1007/978-3-642-30123-0_51

99. Panwichian, S., Kantachote, D., Wittayaweerasak, B., Mallavarapu, M., 2012. The use of selected purple nonsulfur bacteria to remove heavy metals and salts from sediment and water collected from contaminated areas to decrease their phytotoxicity. African Journal of Biotechnology 11, 7434–7444. doi:10.5897/AJB11.3092

100. Panwichian, S., Kantachote, D., Wittayaweerasak, B., Mallavarapu, M., 2011. Removal of heavy metals by exopolymeric substances produced by resistant purple nonsulfur bacteria isolated from

contaminated shrimp ponds. Electronic Journal of Biotechnology 14, 2. doi:10.2225/vol14-issue4-fulltext-2

101. Panwichian, S., Kantachote, D., Wittayaweerasak, B., Mallavarapu, M., 2010. Isolation of purple nonsulfur bacteria for the removal of heavy metals and sodium from contaminated shrimp ponds. Electronic Journal of Biotechnology 13. doi:10.2225/vol13-issue4-fulltext-8

102. Park, J.B.K., Craggs, R.J., 2010. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. Water Science and Technology 61, 633–639. doi:10.2166/wst.2010.951

103. Posadas, E., Bochon, S., Coca, M., 2014. Microalgae-based agro-industrial wastewater treatment : a preliminary screening of biodegradability 2335–2345. doi:10.1007/s10811-014-0263-0

104. Posadas, E., García-Encina, P.A., Domínguez, A., Díaz, I., Becares, E., Blanco, S., Muñoz, R., 2014a. Enclosed tubular and open algal-bacterial biofilm photobioreactors for carbon and nutrient removal from domestic wastewater. Ecological Engineering 67, 156–164. doi:10.1016/j.ecoleng.2014.03.007

105. 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. Bioresource Technology 139, 50–58. doi:10.1016/j.biortech.2013.04.008

106. Posadas, E., Marín, D., Blanco, S., Lebrero, R., Muñoz, R., 2017. Simultaneous biogas upgrading and centrate treatment in an outdoors pilot scale high rate algal pond. Bioresource Technology 232, 133–141. doi:10.1016/j.biortech.2017.01.071

107. Posadas, E., Morales, M.D.M., Gomez, C., Acién, F.G., Muñoz, R., 2015a. Influence of pH and CO2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chemical Engineering Journal 265, 239–248. doi:10.1016/j.cej.2014.12.059

108. Posadas, E., Muñoz, A., García-González, M.-C., Muñoz, R., García-Encina, P., 2014b. A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters. Journal of Chemical Technology and Biotechnology 90, 1094–1101. doi:10.1002/jctb.4417

109. Posadas, E., Serejo, M.L., Blanco, S., Pérez, R., García-Encina, P.A., Muñoz, R., 2015b. Minimization of biomethane oxygen concentration during biogas upgrading in algal-bacterial photobioreactors. Algal Research 12, 221–229. doi:10.1016/j.algal.2015.09.002

110. Prandini, J.M., da Silva, M.L.B., Mezzari, M.P., Pirolli, M., Michelon, W., Soares, H.M., 2016. Enhancement of nutrient removal from swine wastewater digestate coupled to biogas purification by microalgae Scenedesmus spp. Bioresource Technology 202, 67–75. doi:10.1016/j.biortech.2015.11.082

111. Pulz, O., 2001. Photobioreactors: Production systems for phototrophic microorganisms. Applied Microbiology and Biotechnology 57, 287–293. doi:10.1007/s002530100702

112. Puyol, D., Barry, E.M., Hülsen, T., Batstone, D.J., 2017. A mechanistic model for anaerobic phototrophs in domestic wastewater applications: Photo-anaerobic model (PAnM). Water Research 116, 241–253. doi:10.1016/j.watres.2017.03.022

113. Racault, Y., Boutin, C., 2005. Waste stabilisation ponds on France: State of the art and recent trends. Water Science and Technology 51, 1–9.

114. Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2011. Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production.

Applied Energy 88, 3411–3424. doi:10.1016/j.apenergy.2010.11.025

115. Riaño, B., Hernández, D., García-González, M.C., 2012. Microalgal-based systems for wastewater treatment: Effect of applied organic and nutrient loading rate on biomass composition. Ecological Engineering 49, 112–117. doi:10.1016/j.ecoleng.2012.08.021

116. Riaño, B., Molinuevo, B., García-González, M.C., 2011. Treatment of fish processing wastewater with microalgae-containing microbiota. Bioresource Technology 102, 10829–10833. doi:10.1016/j.biortech.2011.09.022

117. Richmond, A., 2004. Handbook of microalgal culture: biotechnology and applied phycology/edited by Amos Richmond. doi:10.1002/9780470995280

118. Rittmann, B.E., McCarty, P.L., 2012. Environmental Biotechnology:Principles and Applications, 1 st. ed. Tata McGraw-Hill, New Delhi.

119. Romera, E., González, F., Ballester, A., Blázquez, M.L., Muñoz, J.A., 2007. Comparative study of biosorption of heavy metals using different types of algae. Bioresource Technology 98, 3344–3353. doi:10.1016/j.biortech.2006.09.026

120. Saavedra, R., Muñoz, R., Taboada, M.E., Vega, M., Bolado, S., 2018. Comparative uptake study of arsenic, boron, copper, manganese and zinc from water by different green microalgae. Bioresource Technology 263, 49–57. doi:10.1016/j.biortech.2018.04.101

121. Safi, C., Zebib, B., Merah, O., Pontalier, P.-Y., Vaca-García, C., 2014. Morphology, composition, production, processing and applications of Chlorella vulgaris: A review. Renewable and Sustainable Energy Reviews 35, 265–278. doi:10.1016/j.rser.2014.04.007

122. Sarkar, N., Ghosh, S.K., Bannerjee, S., Aikat, K., 2012. Bioethanol production from agricultural wastes: An overview. Renewable Energy 37, 19–27. doi:10.1016/j.renene.2011.06.045

123. Seadi, T.A., Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S., Janssen, R., 2008. Biogas Handbook, Igarss 2014. doi:10.1533/9780857097415.1.85

124. Serejo, M.L., Posadas, E., Boncz, M.A., Blanco, S., García-Encina, P., Muñoz, R., 2015. Influence of biogas flow rate on biomass composition during the optimization of biogas upgrading in microalgal-bacterial processes. Environmental Science and Technology 49, 3228–3236. doi:10.1021/es5056116

125. Shriwastav, A., Gupta, S.K., Ansari, F.A., Rawat, I., Bux, F., 2014. Adaptability of growth and nutrient uptake potential of Chlorella sorokiniana with variable nutrient loading. Bioresource Technology 174, 60–66. doi:10.1016/j.biortech.2014.09.149

126. Singh, S.P., Singh, P., 2015. Effect of temperature and light on the growth of algae species: A review. Renewable and Sustainable Energy Reviews 50, 431–444. doi:10.1016/j.rser.2015.05.024

127. statista, 2018. Global pork production in 2017, by country [WWW Document]. URL https://www.statista.com (accessed 5.10.18).

128. Su, Y., Mennerich, A., Urban, B., 2011. Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. Water Research 45, 3351–3358. doi:10.1016/j.watres.2011.03.046

129. Sutherland, D.L., Turnbull, M.H., Craggs, R.J., 2014. Increased pond depth improves algal productivity and nutrient removal in wastewater treatment high rate algal ponds. Water research 53, 271–81. doi:10.1016/j.watres.2014.01.025

130. Talaiekhozani, A., Rezania, S., 2017. Application of photosynthetic bacteria for removal of heavy metals, macro-pollutants and dye from wastewater: A review. Journal of Water Process Engineering 19, 312–321. doi:10.1016/j.jwpe.2017.09.004

131. Talbot, P., Thébault, J.M., Dauta, A., De la Noüe, J., 1991. A comparative study and mathematical modeling of temperature, light and growth of three microalgae potentially useful for wastewater treatment. Water Research 25, 465–472. doi:10.1016/0043-1354(91)90083-3

132. Tang, K.H., Tang, Y.J., Blankenship, R.E., 2011. Carbon metabolic pathways in phototrophic bacteria and their broader evolutionary implications. Frontiers in Microbiology 2, 1–23. doi:10.3389/fmicb.2011.00165

133. Tijani, H., Abdullah, N., Yuzir, A., 2015. Integration of microalgae biomass in biomethanation systems. Renewable and Sustainable Energy Reviews 52, 1610–1622. doi:10.1016/j.rser.2015.07.179

134. Toledo-Cervantes, A., Estrada, J., Lebrero, R., Muñoz, R., 2017a. A comparative analysis of biogas upgrading technologies: photosynthetic vs physical/chemical processes. Algal Research In review, 237–243. doi:10.1016/j.algal.2017.05.006

135. Toledo-Cervantes, A., Madrid-Chirinos, C., Cantera, S., Lebrero, R., Muñoz, R., 2017b. Influence of the gas-liquid flow configuration in the absorption column on photosynthetic biogas upgrading in algal-bacterial photobioreactors. Bioresource Technology 225, 336–342. doi:10.1016/j.biortech.2016.11.087

136. Toledo-Cervantes, A., Serejo, M.L., Blanco, S., Pérez, R., Lebrero, R., Muñoz, R., 2016. Photosynthetic biogas upgrading to bio-methane: Boosting nutrient recovery via biomass productivity control. Algal Research 17, 46–52. doi:10.1016/j.algal.2016.04.017

137. Tsuyuzaki, S., 2014. http://hosho.ees.hokudai.ac.jp [WWW Document]. Biological and physical cycle in nature. URL http://hosho.ees.hokudai.ac.jp/~tsuyu/top/dct/bgc.html (accessed 6.8.18).

138. UN WWAP, 2017. WWAP (United Nations World Water Assessment Programme).

139. United Nations, 2015. World Populations Prospects: The 205 Revision; Volume II: Demographic Profiles (st/esa/ser.a/380). New York, USA.

140. United States Environmental Protection Agency, 2013. Wastewater treatment fact sheet : external carbon sources for nitrogen removal 5.

141. Unnithan, V. V., Unc, A., Smith, G.B., 2014. Mini-review: A priori considerations for bacteria–algae interactions in algal biofuel systems receiving municipal wastewaters. Algal Research 4, 35–40. doi:10.1016/j.algal.2013.11.009

142. UNWater, 2015. Water for a sustainable world Water for a sustainable.

143. Vivek, Gupta, A.K., 2004. Biodiesel production from Karanja oil. Journal of Scientific and Industrial Research 63, 39–47.

144. von Sperling, M., 2007. Wastewater characteristics, treatment and disposal, Choice Reviews Online. doi:10.5860/CHOICE.45-2633

145. Wang, M., Yang, H., Ergas, S.J., van der Steen, P., 2015. A novel shortcut nitrogen removal process using an algal-bacterial consortium in a photo-sequencing batch reactor (PSBR). Water Research 87, 38–48. doi:10.1016/j.watres.2015.09.016

146. Wang, Z., Wen, X., Xu, Y., Ding, Y., Geng, Y., Li, Y., 2018. Maximizing CO2 biofixation and lipid productivity of oleaginous microalga Graesiella sp. WBG-1 via CO2-regulated pH in indoor and

outdoor open reactors. Science of the Total Environment 619–620, 827–833. doi:10.1016/j.scitotenv.2017.10.127

147. Wayne Chew, K., Ying Yap, J., Loke Show, P., Hui Suan, N., Ching Juan, J., Chuan Ling, T., Lee, D.-J., Chang, J.-S., 2017. Microalgae biorefinery: high value products perspectives. Bioresource Technology 229, 53–62. doi:10.1016/j.biortech.2017.01.006

148. Xia, A., Murphy, J.D., 2016. Microalgal Cultivation in Treating Liquid Digestate from Biogas Systems. Trends in Biotechnology 34, 264–275. doi:10.1016/j.tibtech.2015.12.010

149. Xu, J., Zhao, Y., Zhao, G., Zhang, H., 2015. Nutrient removal and biogas upgrading by integrating freshwater algae cultivation with piggery anaerobic digestate liquid treatment. Applied Microbiology and Biotechnology 99, 6493–6501. doi:10.1007/s00253-015-6537-x

150. Yang, J.S., Cao, J., Xing, G.L., Yuan, H.L., 2015. Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae Chlorella minutissima UTEX2341. Bioresource Technology 175, 537–544. doi:10.1016/j.biortech.2014.10.124

151. Yuan, Z., Pratt, S., Batstone, D.J., 2012. Phosphorus recovery from wastewater through microbial processes. Current Opinion in Biotechnology 23, 878–883. doi:10.1016/j.copbio.2012.08.001

152. Yurkov, V. V., Beatty, J.T., 1998. Aerobic Anoxygenic Phototrophic Bacteria. Microbiol. Mol. Biol. Rev. 62, 695–724. doi:10.1038/415630a.

153. Zhang, D., Yang, H., Zhang, W., Huang, Z., 2003. Rhodocista pekingensis sp . nov ., a cyst-forming phototrophic bacterium from a municipal wastewater treatment plant. International Journal of Systematic and Evolutionary Microbiology 53, 1111–1114. doi:10.1099/ijs.0.02500-0

Aims and Scope

2.1 Justification

During the past decades, the intense growth of human population has generated large amounts of domestic and livestock wastewaters, which must be treated before discharge into water bodies. In this context, multiple biological and physical/chemical technologies have been engineered to treat wastewater. Algal-bacterial processes have received an increasing attention as a cost-efficient platform for water reclamation as a result of their low-cost oxygenation, simplicity, nutrient recovery potential, carbon dioxide mitigation potential and production of a valuable feedstock biomass. This solar driven technology is based on the combination of heterotrophic, mixotrophic and phototrophic metabolisms and UV photolysis, and can be coupled with biogas upgrading. However, this green technology only exhibits consistent C, N and P removal efficiencies when treating effluents with high C/N/P ratios (≈100/18/1), which are not typically encountered in domestic and livestock wastewaters. Despite the advantages of algal-bacterial processes in wastewater treatment, more research is needed to optimize wastewater treatment and to guarantee a consistent biomass composition in order to support the development of algae-based biorefineries for the production of biofuels, biofertilizers or high value bioproducts. On the other hand, purple photosynthetic bacteria (PPB) have emerged as an alternative photosynthetic platform for wastewater treatment based on their superior resource recovery potential, higher tolerance to organic pollution, lower dependence to temperature and more versatile metabolism. In brief, microalgae and PPB based wastewater treatment has the potential to provide a simple, low-cost and environmentally friendly solution for wastewater sanitation in developing countries, where environmental and social conditions are more favorable to these solar based technologies and the acceptance of the bioproducts generated.

This thesis aimed at tackling the main problems hindering the widespread implementation of algal-bacterial photobioreactors during wastewater treatment: i) the low concentration of inorganic carbon in domestic wastewater, ii) the lack of studies confirming the potential of denitrification-nitrification of algal-bacterial photobioreactors using real domestic wastewater, iii) the poor knowledge of the dynamics of the microalgae population structure in algal-bacterial photobioreactors, iv) the representativeness of the results obtained indoors and v) the lack of alternatives to microalgae as a platform for photosynthetic wastewater treatment.

2.2 Objectives

The overall goal of the present thesis was the assessment of the potential of photosynthetic treatment of domestic and livestock wastewater using algal-bacterial consortia and purple photosynthetic bacteria. The work conducted was focused on evaluating the potential of novel bioreactor configurations and photosynthetic microorganisms, and providing insights on the long-term feasibility of alga biorefineries based on wastewater treatment. More specifically:

Objective 1. Systematic evaluation of the performance of novel photobioreactor configurations capable of overcoming the limitations of conventional High Rate Algae Ponds during domestic wastewater treatment.

Objective 2. Evaluation of the long-term dominance of specific microalgae and cyanobacteria during piggery wastewater treatment.

Objective 3. Evaluation of relevance of the results obtained indoors under artificial irradiation.

Objective 4. A comparative evaluation of the performance of algal-bacterial consortia and purple photosynthetic bacteria.

2.3 Development of the thesis

In order to fulfill the specific objectives above stated, four series of experiments were conducted over the past 4 years:

The performance of an innovative anoxic-aerobic photobioreactor coupled with biogas upgrading and operated with biomass recycling was assessed (**Chapter 1**).
In this context:

- ✓ This work was devised to treat urban domestic wastewater with low inorganic carbon concentration at short hydraulic retention times.
- ✓ Biogas addition was used to overcome inorganic carbon limitation and promote a stable nitrification-denitrification process.
- ✓ The process incorporated a biomass settling step followed by recycling to the anoxic tank to promote a total denitrification and improve the biomass settling characteristics.

The long-term dynamics of the structure of microalgae population and wastewater treatment performance was evaluated during piggery wastewater treatment in open photobioreactors (**Chapter 2**).

- ✓ This study was carried out to estimate microalgae production at large-scale in the context of microalgae-based biorefineries.
- ✓ Piggery wastewater was used as a nutrient source due to the high organic matter and nutrients concentrations, able to support high biomass productivities.
- ✓ The dynamics of microalgae population structure were evaluated in four photobioreactors under similar operational conditions and inoculated with two microalgae species, a cyanobacterium and compared with a system not inoculated.

Piggery wastewater (PWW) treatment in open algal-bacterial photobioreactors was also comparatively evaluated under indoor and outdoor conditions (Chapter **3**).

- ✓ A comparative study was carried to evaluate microalgae evolution and process performance in both operational conditions, since most studies have been conducted under laboratory conditions.

- ✓ Two PWW dilutions were used in order to evaluate the tolerance to organic pollution and its influence on microalgae dominance during PWW treatment.

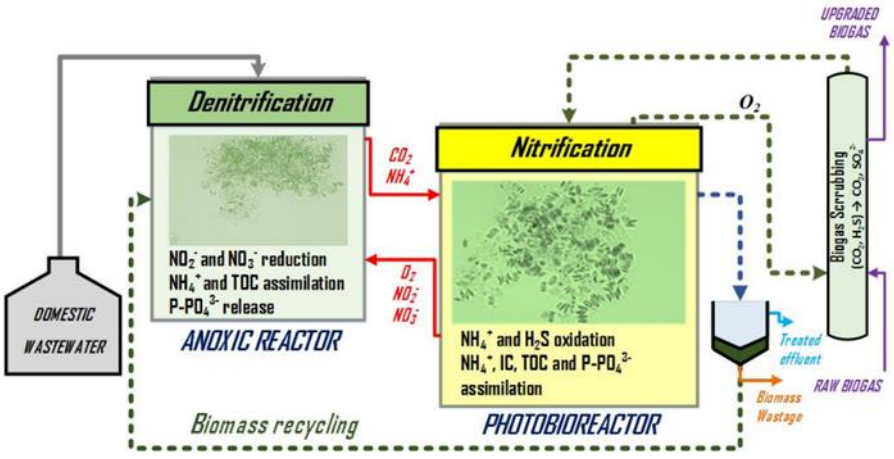
Finally, the potential of purple photosynthetic bacteria for piggery wastewater treatment under infrared illumination was comparatively tested against that of an algal-bacterial consortium under LED illumination in open photobioreactors under indoor conditions (**Chapter 4**).

- ✓ This study was conducted to elucidate the best photosynthetic platform during PWW treatment.
- ✓ The performance of both microbial groups at different hydraulic retention times was evaluated both in term of biomass productivity, effluent quality and potential for nutrient recovery.

Chapter 1

Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading

García, D., Alcántara, C., Blanco, S., Pérez, R., Bolado, S., Muñoz, R. 2017. Chemical Engineering Journal. 313, 424 - 434. doi:10.1016/j.cej.2016.12.054. Accepted 13 December 2016



Enhanced Carbon, Nitrogen and Phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading

Dimas García[1], Cynthia Alcántara[1], Saúl Blanco[2], Rebeca Pérez[1], Silvia Bolado[1], Raúl Muñoz[1]*

1- Department of Chemical Engineering and Environmental Technology, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

2- The Institute of the Environment, La Serna 58 - 24007 León, Spain

* Corresponding author: mutora@iq.uva.es

Abstract

This work evaluated the performance of an innovative anoxic-aerobic algal-bacterial photobioreactor coupled with biogass upgrading for the treatment of domestic wastewater via nitrification-denitrification. The process, which incorporated a biomass settling step followed by recycling to the anoxic tank, was operated at a hydraulic retention time of 2 days, a sludge retention time of ≈11 days under a 12h/12h light/dark irradiation cycle at 392 μE $m^{-2}·s^{-1}$. An increase in the removal efficiency of TN from 38% to 81%, NH_4^+ from 39% to 97%, and P-PO_4^{3-} from 59% to 64% were recorded when additional CO_2 was supplied to the photobioreactor via biogas scrubbing, which supported an almost complete nitrification of the NH_4^+ to NO_3^- and promoted microalgae growth (with the subsequent enhancement in N and P assimilation). TOC removal remained constant at 90±2% regardless of the addition of CO_2, while the effluent biomass concentration averaged 26±12 mgTSS/L. A DGGE-sequencing analysis of the bacterial community revealed the occurrence of 10 phyla, *Proteobacteria* being the dominant phylum. Finally, the morphological characterization of the microalgae population dynamics revealed a gradual dominance of the genus *Scenedesmus*, which accounted for 94-100 % at the end of the experiment.

Keywords

Biogas upgrading;
Biomass recycling;
Inorganic carbon limitation;
Microalgae;
Nitrification-denitrification.

1. Introduction

The steady increase in human population [1] and industrial activity is generating large amounts of wastewaters and greenhouse gases [2], which represent two of the major environmental challenges to global sustainability nowadays. Domestic and industrial wastewaters and anaerobic digestion effluents are characterized by their high loads of carbon (C), nitrogen (N) and phosphorus (P), which must be treated before discharge into natural water bodies to avoid oxygen depletion, toxicity issues and eutrophication [3]. A wide range of biological and physical/chemical technologies is currently available for carbon and nutrient removal in wastewater treatment plants (WWTPs). Unfortunately, these technologies often entail high investment and operating costs and do not allow for a cost-effective recovery of nutrients due to the low C/N and C/P ratios of most domestic and industrial wastewaters [4][5].

In this context, algal-bacterial processes can support both a low-cost process oxygenation and an enhanced nutrient recovery. The oxygen produced by microalgae during photosynthesis can support the oxidation of organic pollutants and ammonium by aerobic heterotrophs and nitrifiers, respectively, which thus reduces the operating costs and environmental impacts associated with conventional mechanical aeration in WWTPs [6]. On the other hand, the ability of algal-bacterial consortia to assimilate both organic carbon (inherently present in most wastewaters) and inorganic carbon (from the biological oxidation of organic carbon, alkalinity in wastewater or residual carbon dioxide (CO_2) externally supplied) result in larger biomass productivities and therefore enhanced nutrient recoveries[7]. However, despite the above-mentioned advantages, algal-bacterial processes devoted to wastewater treatment still present severe technical limitations that hinder the full-scale implementation of this technology, such as nutrient supply and recycling, gas transfer and exchange [8].

In this regard, although photoautotrophic algal metabolism can enhance N and P recovery in anoxic-aerobic algal-bacterial photobioreactor (AA-ABPh), the alkalinity present in raw wastewaters is low to support a complete nutrient recovery/removal and residual CO_2 sources (such as flue gas) are not always available on-site. In addition, the low hydraulic retention times (HRT)

required in algal-bacterial processes to compete with activated sludge systems would limit the development of nitrifying bacterial communities that would eventually support nitrification-denitrification processes during the treatment of wastewaters with low C/N ratios. Finally, the poor sedimentation ability of the microalgae generated in the process often results in effluent total suspended solid concentrations (TSS) above the maximum European Union (EU) discharge limit (50 mg/L), which limits the scale-up of microalgae-based wastewater treatment [9]. In this context, AA-ABPh operated with autofloculated biomass settling and recycling constitutes an innovative technology capable of overcoming the above mentioned limitations. This technology was successfully evaluated for the treatment of synthetic wastewaters at moderate HRTs but experienced severe inorganic carbon limitations, which ultimately restricted the treatment potential of this innovative technique. Therefore, there is an urgent need to develop novel operating strategies to overcome the above mentioned inorganic carbon limitation and to evaluate the performance of this innovative technology using real domestic wastewater (RDWW) at low HRTs.

This work was devised to evaluate the treatment of RDWW in an innovative AA-ABPh configuration operated with biomass settling and recycling at a HRT of 2 days coupled to the simultaneous upgrading of synthetic biogas (in a separate and interconnected column). In this system, the supply of biogas (eventually available on-site from the anaerobic digestion of the algal-bacterial biomass produced in the process) will provide the additional inorganic C source required to boost nutrient removal by assimilation and bacterial nitrification to sustain an efficient nitrification-denitrification process [10][11]. The influence of photosynthetic biogas upgrading on the mechanisms underlying C, N and P removal in the anoxic tank and photobioreactor treating RDWW was assessed using a mass balance approach. A detailed characterization of the dynamics of microalgal and bacterial population structure was conducted using morphological and molecular identification tools.

2. Materials and methods

2.1 Microorganisms and culture conditions

The anoxic and aerobic tanks were inoculated with 3.2 g/L of total

suspended solid (TSS) of an mixture of a microalgal-cyanobacterial consortium (from now on referred to as microalgae) from a high rate algal ponds (HRAP) treating diluted vinasse [12] and aerobic activated sludge from Valladolid WWTP (Spain). Domestic wastewater was collected from a nearby sewer located at Department of Chemical Engineering and Environmental Technology of Valladolid University. The average composition of the RDWW treated continuously was: 176±26 mg/L of dissolved total organic carbon (TOC), 152±34 mg/L of dissolved inorganic carbon (IC), 106±9 mg/L of total nitrogen (TN), 93±9 mg/L of N-ammonium ($N\text{-}NH_4^+$), 39±12mg/L of sulfate (SO_4^{2-}) and 33±8 mg/L of P-phosphate ($P\text{-}PO_4^{3-}$) (Table 1).

2.2 Experimental set-up

Three operational stages (SI, SII and SIII) were carried out in the experimental set-up below described to evaluate the influence of biogas supply and internal recycling rate on WWT performance. The experimental set-up consisted of an anoxic tank, with a total working volume of 0.9 L, interconnected to a photobioreactor with a total working volume of 2.7 L. Both reactors were operated as completely mixed flow reactors. The anoxic tank was maintained in the dark and magnetically stirred at 320 rpm. The photobioreactor was continuously illuminated for 12 hours/day (05:00h to 17:00h) by LED lamps arranged in a concentric configuration providing an average light intensity of 392±19 $\mu mol/m^2 \cdot s$ at the outer wall of the photobioreactor (Figure 1). Air was introduced during the dark period in order to maintain the dissolved O_2 concentration in the photobioreactor above non-limiting concentrations [13][14] (Table 1). The temperature in the jacketed photobioreactor was maintained constant at 25±1°C using a recirculating water stream cooled down in a water bath (Huber, Germany). The temperature in the photobioreactor was controlled to systematically assess the influence of biogas supply and internal recirculation flow rate on process performance without the interference of any other varying parameter. In this context, the 12h/12h light/dark illumination regime would entail an increase in process temperature (as a result of the heat dissipated from the LEDs) during the illuminated period that might interfere with the activity of the most sensitive microbial communities (i.e nitrifiers). The temperature in the anoxic tank was not controlled but remained constant at 25 ºC as a result of the constant room temperate and high

internal recycling flow rate from the photobioreactor. Likewise, magnetic agitation in the photobioreactor was maintained constant and 320 rpm. During stage II (SII) and stage III (SIII), the photobioreactor was interconnected to a 0.3 L external absorption column (Ø = 2.54 cm; height = 60 cm) in order to provide an extra source of CO2 via photosynthetic biogas upgrading (Figure 1). The use of a separate biogas absorption column supported both a higher CO_2 gas-liquid mass transport and a lower O_2 stripping. Hence, the direct scrubbing of biogas in the typically shallow photobioreactors would entail a low volumetric CO_2 gas-liquid mass transfer coefficient, while an external absorption column can be designed independently of the light path (depth) of the photobioreactor. On the other hand, process operation with an external absorption column interconnected to the HRAP with a recycling cultivation broth stream allows a control of the oxygen potentially stripped out to the upgraded biogas.

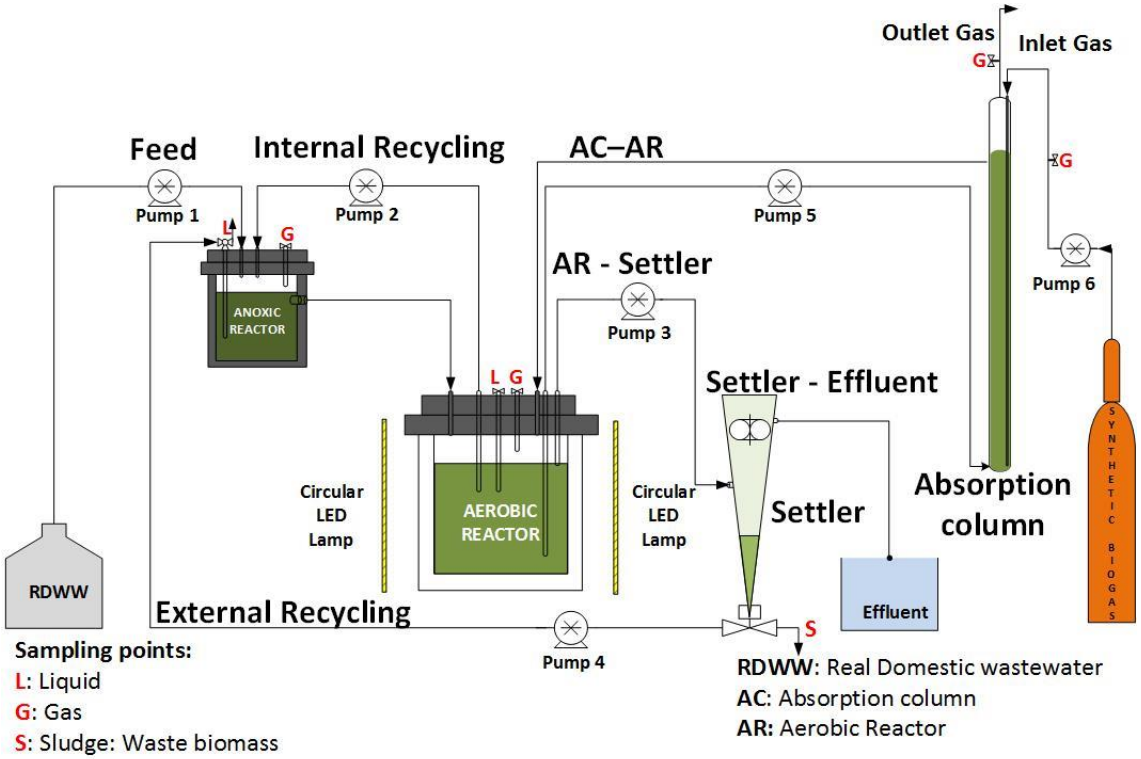


Figure 1. Schematic diagram of the anoxic-aerobic algal-bacterial photobioreactor set-up coupled with an absorption column for CO2 supplementation via biogas upgrading.

2.3 Experimental design

The HRT and the Sludge Retention Time (SRT) were maintained at 2 days (HRT_{anoxic} = 0.5 day, $HRT_{aerobic}$ = 1.5 days) and ≈ 11 days, respectively, during the whole experiment (Table 1). These

short HRTs are required to make algal-bacterial processes competitive with activated sludge systems. The experimental design was based on the operational limitations identified in previous studies in this AA-ABPh configuration [13][14]. The experiment was divided in three operational stages.

The internal recycling (IR) flow rate from the photobioreactor to the anoxic tank was maintained at 2.8 L/d during SI (maintained for 78 days) and SII (maintained for 74 days), while this parameter was increased by 30% (3.6 L/d) during SIII (maintained for 56 days) in order to evaluate the maximum denitrifying capacity in the anoxic tank to ultimately boost the dissimilatory N removal in the experimental system. The external recycling flow rate (ER) from the settler to the anoxic tank was maintained at 0.5 L/d during the three operational stages (Table 1). The pH in the photobioreactor was maintained between 7.0 and 8.6 during SI by daily addition of 1.2 mL of chlorhydric acid (37%), while biogas upgrading into the absorption column supplied CO_2 to overcome the IC limitation encountered during SI and to maintain the pH below inhibitory values for bacterial activity (< 9) during SII and SIII. The synthetic biogas mixture supplied was composed of methane (70%), carbon dioxide (29.5%) and hydrogen sulfide (0.5%) (Abello Linde, Barcelona, Spain). Biogas was supplied to the absorption column at 2.6 L/d (1.8 ml/min) through a 10 µm metallic sparger located at the bottom of the column co-currently with a recycling algal-bacterial broth stream drawn from the photobioreactor at a liquid to biogas ratio (L/G) of 10 (v/v) (Figure 1). Liquid samples of 100 ml were taken twice per week from the RDWW, anoxic tank, aerobic tank, settled biomass and effluent to determine concentrations of TOC, IC, TN, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, SO_4^{2-}, $P\text{-}PO_4^{3-}$ and TSS. pH, temperature and dissolved oxygen concentration (DO) were measured daily in both bioreactors. Likewise, the C, N and P content of the algal bacterial biomass was measured under steady state at each operational stage. The sludge volumetric index (SVI) and the maximum biomass settling rate, which were used to monitor the settling characteristics of the algal-bacterial biomass [15], were determined in the anoxic and aerobic bioreactors under steady state at each operational stage. The microalgae population structure was assessed at the end of SI, SII and SIII using biomass samples from the photobioreactor preserved with lugol acid at 5% and formaldehyde at 10%,

and stored at 4 ºC prior to analysis. Biomass samples from the photobioreactor were collected at the end of each steady state, and immediately stored at -20 ºC in order to evaluate the richness and composition of the bacterial communities [14]. Finally, the composition of the biogas at the inlet and outlet of the absorption column was determined twice a week during SII and SIII.

2.4 Mass balance

The dilution effect in the anoxic tank caused by the internal and external recirculations was considered by calculating a virtual concentration for each parameter in the influent wastewater to the anoxic tank. Hence, the actual C, N and P removals in the denitrification reactor were assessed using the virtual concentrations (V*i*) for dissolved IC, TOC, N-NH_4^+ and P-PO_4^{3-} at the entrance of the anoxic tank according to Eq. (1):

$$V_i\left(\frac{mg}{L}\right) = \frac{(C_{i\,feed}\cdot Q_{feed})+(C_{i\,aerobic}\cdot Q_{RI})+(C_{i\,aerobic}\cdot Q_{RE})}{Q_{feed}+Q_{RI}+Q_{RE}} \quad (1)$$

where $C_{i\ feed}$ and $C_{i\ aerobic}$ represent the dissolved concentrations of the parameter "*i*"= TOC, IC, TN, N-NH_4^+ and P-PO_4^{3-} in the RWW and the photobioreactor, respectively, while Q_{feed} represents the RDWW flow rate, Q_{RI} the internal recirculation flow rate and Q_{RE} the external recirculation flow rate.

An overall mass balance to the anoxic-aerobic photobioreactor was conducted for TOC, IC, TN, N-NH_4^+ and P-PO_4^{3-} based on their average concentrations under steady state conditions for each operational stage. The validity of the instrumental and analytical methods was thus assessed by means of the mass recovery factors for the parameter "*i*" according to Eq. (2) [14]:

$$M_i\ Recovery\ (\%) = \frac{(M_{i\,rem})\boldsymbol{anox}+(M_{i\,rem})\boldsymbol{photobior}+(M_i)\boldsymbol{effl}}{(M_i)RWW}\cdot 100 \quad (2)$$

where $(M_{i\ rem})_{anoxic}$ and $(M_{i\ rem})_{photobior}$ represent the mass flow rate (g/d) of the parameter ***i*** = TOC, IC, TN, N-NH_4^+ and P-PO_4^{-3} removed in the anoxic tank and photobioreactor, respectively. $M_{i\ effl}$ and $M_{i\ RWW}$ represent the mass flow rate (g/d) of the parameter in the treated effluent and RWW, respectively.

The removal efficiencies herein reported for each tank refer to the individual contribution of the anoxic and photobioreactor units to the overall removal of the inlet loading for each monitored parameter.

2.5 Analytical procedures

The light intensity was measured as photosynthetically active radiation (PAR) using a LI-250A light meter (LI-COR Biosciences, Germany). Biogas composition was determined using a Bruker 430 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. The injector, detector and oven temperatures were maintained at 150 ºC, 175 ºC and 40 ºC, respectively. Helium was used as the carrier gas at 13.7 cm^3/min[16]. TOC, IC and TN concentrations were measured using a TOC-V CSH analyzer equipped with a TNM-1 module (Shimadzu, Japan). N-NH_4^+ was measured using the Nessler analytical method [15] in a U-2000 spectrophotometer (Hitachi, Japan), while NO_2^- and NO_3^- were determined by the cadmium reduction column method [15]. P-PO_4^{-3} and SO_4^{2-} were analyzed by high performance liquid chromatography-ion chromatography (HPLC-IC) with a Waters 515 HPLC pump coupled with a Waters 432 ionic conductivity detector and equipped with an IC-Pak Anion HC (150 mm × 4.6 mm) waters column. A 510 pH meter (EUTECH Instrument, The Netherlands) was used for pH determination. DO concentration and temperature were recorded using an OXI 330*i* oximeter (WTW, Germany). The determination of the TSS concentration, SVI and settling rate were conducted according to Standard Methods [15]. The analysis of the C and N biomass content was carried out using a LECO CHNS-932 elemental analyzer with pre-dried and grinded algal-bacterial biomass. The content of P in the biomass was measured using a 725-ICP Optical Emission Spectrophotometer (Agilent, USA) at 213.62 nm. The identification, quantification and biometry measurements of microalgae were conducted by microscopic examination (OLYMPUS IX70, USA) of the algal-bacterial cultivation broths according to Phytoplankton Manual [17].

Genomic DNA was extracted using the protocol described in the Fast® DNA Spin Kit for Soil (MP Biomedicals, LLC) handbook. The V6-V8 regions of the bacterial 16S ribosomal ribonucleic acid (rRNA) genes were amplified by Polymerase Chain Reaction (PCR)

analysis using the universal bacterial primers 968-F-GC and 1401-R (Sigma-Aldrich, St. Louis, MO, USA; [18]). The PCR mixture contained 1 µL of each primer (10 ng µL-1 each primer), 25 µL of BIOMIX ready-to-use 2 reaction mix (Bioline, Ecogen), 2 µL of the extracted DNA and Milli-Q water up to a final volume of 50 µL. The PCR thermo-cycling program consisted of 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. The denaturing gradient gel electrophoresis (DGGE) analysis of the amplicons was performed with a D-Code universal mutation system (Bio Rad Laboratories) using 8% (w/v) polyacrylamide gels with a urea/formamide denaturing gradient of 45 to 65%. DGGE running conditions were applied according to Roest et al. (2005) [19]. The gels were stained with GelRed Nucleic Acid Gel Stain (biotium) for 1 h. The most relevant bands were excised from the DGGE gel in order to identify the bacteria present in the samples, resuspended in 50 µL of ultrapure water and maintained at 60 °C for 1hour to allow DNA extraction from the gel. A volume of 5 µL of the supernatant was used for reamplification with the original primer set. Before sequencing, PCR products were purified with the GenElute PCR DNA Purification Kit (Sigma-Aldrich, St. Louis, MO, USA). DGGE profiles were compared using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). After image normalization, bands were defined for each sample using the bands search algorithm within the program. The peak heights in the densitometric curves were also used to determine the Shannon-Wiener diversity index (H). Therefore, this index reflects both the sample richness (relative number of DGGE bands) and evenness (relative intensity of every band). It ranges from 1.5 to 3.5 (low and high species evenness and richness, respectively) and can be calculated according to Eq. (3)[20]:

$$H = -\Sigma\,[P_i ln(P_i)] \qquad (3)$$

Where H is diversity index and P_i is the importance probability of the bands in a lane ($P_i = n_i/n$, where n_i is the height of an individual peak and n is the sum of all peak heights in the densitometric curves). Similarity indices of the compared profiles were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient [21].The taxonomic position of the

sequenced DGGE bands was obtained using the RDP classifier tool (50% confidence level) [22]. The closest cultured and uncultured relatives to each band were obtained using the BLAST search tool at the NCBI (National Centre for Biotechnology Information) [23]. The sequences generated from this work are deposited in GenBank under accession numbers KU854389-KU854421.

2.6 Statistical analysis

The data displayed in Table 1, Figure 2 and Figure 7(c) correspond to the mean ± standard deviation of the target parameters during steady state at each operational stage. A one-way ANOVA analysis was performed to assess any significant difference between the settling rate of the biomass from the anoxic reactor and the photobioreactor using Excel (Microsoft, USA). A Pearson correlation analysis was conducted to determine the similarity indexes among the population established during steady state operation.

Table 1. Operational conditions and physical/chemical characterization of the real wastewater and cultivation broth in the anoxic tanks and photobioreactor.

Stage		**Wastewater**	**SI**		**SII**		**SIII**	
Parameter / Reactor			**Anoxic**	**Aerobic**	**Anoxic**	**Aerobic**	**Anoxic**	**Aerobic**
Operational period (days)		n.a	78		74		56	
HRT (days)		n.a	0.5	1.5	0.5	1.5	0,5	1.5
SRT (days)		n.a	12.5 ± 3.5		11 ± 0.9		10.5 ± 0.5	
Light (µmol/m^2.s)		n.a	n.a	367±57	n.a	412±15	n.a	395±21
RWW feeding rate (L/d)		n.a	1.8		1.8		1.8	
Internal recycling rate (L/d)		n.a	2.8		2.8		3.6	
External recycling rate (L/d)		n.a	0.5		0.5		0.5	
pH (units)	***Light***	n.a	7.4±0.3	8.6±0.6	6.9±0.1	8.0±0.7	6.8±0.2	8.9±0.9
	Dark	n.a		7.1±0.3		7±0.2		7.0±0.4
Dissolved oxygen (mg/L)	***Light***	n.a	0	23.3±4.1	0	22.0±2.0	0	23.0±1.8
	Dark	n.a		6.0±0.6		3.1±1.2		3.9±0.7
TOC (mg/L)		176±26	26±5	20±4	30±4	19±1	23±2	18±2
IC (mg/L)		152±34	48±5	2±1	56±9	15±5	47±4	13±4
TN (mg/L)		106±9	74±10	66±8	32±4	21±3	28±2	18±4
N-NH_4^+ (mg/L)		93±9	64±4	54±8	32±5	3±1	33±2	3±1
N-NO_2^- (mg/L)		n.a	0.01±0.01	5.6±4.0	0.03±0.05	3.1±3.8	0.41±0.68	1.1±1.8
N-NO_3^- (mg/L)		n.a	0.04±0.03	0.9±0.9	0.14±0.15	8.9±5.5	0.73±1.25	13.0±3.2
P-PO_4^{3-} (mg/L)		33±8	26±5	16±7	18±3	9±2	18±1	11±2
SO_4^{2-}		39±12	29±7	32±6	51±15	76±11	47±7	55±4
TSS (mg/L)		n.a	1519±252	1216±260	3113±361	2854±324	2480±309	2047±186
SVI (mL/g)		n.a	95	161	128	169	80	97
Air flow (mL/min)		n.a	0	6	0	4	0	2
Biogas (L/day)		n.a	n.a			2.6		2.6
n.a : Not applicable								

3. Results and Discussion

The mass balance calculations over the 208 days of operation showed recoveries for TOC, IC, TN and P-PO_4^{3-} of 100±1%, 99±4%, 100±5% and 100±14%, which validated the analytical and instrumental methodologies used in this study. This mass balance approach allowed to better understand the symbiosis between microalgae and bacteria in this novel anoxic-aerobic algal-bacterial photobioreactor configuration [14], by quantifying the extent of the mechanisms involved in C, N and P removal in each reactor.

The overall removal efficiency of organic matter measured as TOC under steady state operation averaged 89±2% along the 3 operational stages at 2 days of HRT due to the high photosynthetic oxygenation capacity and denitrification activity of the system. In this context, while the DO concentration in the anoxic tank remained close to 1 mg O_2/L (thus supporting an efficient denitrification since the O_2 carried out by the internal recycling was lower that O_2 demand of the RDWW), the DO in the photobioreactor fluctuated from 15 and 32 mg O_2/L during illuminated periods to 1.5 and 7 mg O_2/L during dark periods in the photobioreactor (supplementary material Figure S1). These oxygen concentrations were sufficient to satisfy the bacterial demand from NH_4^+ and TOC oxidation in the photobioreactor. The organic matter removal recorded in this study was similar to that typically achieved in conventional activated sludge systems (COD-REs of 85-90%) and in conventional HRAPs treating domestic wastewater (COD-REs 81-88%). The high light intensity used in this lab-scale study to boost microalgae photosynthetic activity (392±19 μmol/m^2·s) supported an efficient overall steady state IC removal (95±4%) mainly based on microalgae assimilation, nitrification representing a minor IC removal mechanism (≈4.1% of the total IC input).

On the other hand, the average TN removal during SI under steady state operation accounted for 38±6% with average NH_4^+ removals of 39±9%. This low TN-RE was due to the low efficiency of NH_4^+ nitrification during SI as a result of a severe IC limitation. Biogas supplementation in SII and SIII overcame this limitation and promoted steady state removals of TN and NH_4^+ of 81±3%, 97±2%, respectively, at a HRT of 2 days. NH_4^+ nitrification in the photobioreactor was the key step to ensure an efficient nitrogen removal in

the anoxic tank via denitrification, despite NH_4^+ oxidation during SI was limited by the active photosynthetic IC uptake by microalgae. Comparable TN-REs ranging from 68% to 85% and N-NH_4^+-REs of 80-93% are typically achieved in CO_2-supplemented HRAPs treating domestic wastewater but at HRTs of 3-7 days, with nitrogen assimilation into biomass and NH_3 stripping identified as the main nitrogen removal mechanisms [24]. Lower TN-REs ranging from 57% to 73% are often achieved in HRAPs during the treatment of domestic sewage without CO_2 supplementation at HRTs of 3-10 days, which highlights the superior performance of our two-stage photobioreactor [25]. In addition, the values hereby obtained for nitrogen removal were comparable with the removal efficiencies of ≈ 80% typically reported in nitrification-denitrification activated sludge plants, although conventional WWTPs operate at 0.5-1 day of HRT [26].

Finally, average orthophosphate removal efficiencies of 59±17% were recorded under steady state operation in SI. However, the supplementation of biogas resulted in an enhanced biomass growth and therefore in a slight increase in P-PO_4^{3-}-REs up to steady state values of 67±13% and 60±6% in SII and SIII, respectively. Bioassimilation into algal-bacterial biomass was likely the main phosphorous removal mechanism since pH values fluctuated from 6.8 to 9.4 during illuminated periods and from 6.4 to 8.1 during the dark periods in the photobioreactor (supplementary material Figure S1). The average pH values recorded along the entire experiment were likely not sufficient to support phosphate precipitation, which has been shown to occur at pHs > 9.0 [6][27]. The P-PO_4^{3-}-REs here obtained (59 - 67%) were similarly those typically reported in HRAPs (50% to 75%) at significantly higher HRTs (3 – 7 days) and activated sludge processes at HRTs of 0.5 - 1 days [24]. However, the volumetric PO_4^{3-} removal rates achieved were superior based on the fact that the phosphate concentration in the RWW used in this study (33±8 mg P/L) was ≈ 5 times higher than the P-PO_4^{3-} concentrations typically present in medium strength WW (≈ 7 mg P/L)[28] (Table 1).

Finally, the high robustness of this process configuration should be highlighted based on the consistent effluent concentrations of TOC, IC, NH_4^+, TN, PO_4^{3-} despite the inherent variations of these parameters in RDWW.

3.1 Carbon and nutrient removal in the anoxic reactor

The overall removal efficiencies of TOC in the anoxic reactor accounted for 77±4% under steady state operation, with values of 77±4%, 76±6% and 79±3% for SI, SII and SIII, respectively (Figure 2a). This heterotrophic TOC removal (organic matter acting as electron donor) resulted in steady state concentrations of 27±5 mg/L in the anoxic tank regardless of the operational stage [29]. On other hand, a negative IC removal efficiency of -14±13% was recorded during SI as a result of CO_2 production from TOC oxidation in the anoxic tank (mainly driven by the use of O_2 as electron acceptor, which represented 67% of the total e^- acceptor consumption during SI) and the absence of a significant CO_2 stripping due to the overall CO_2 limitation in the process (Figure 2b). The slightly higher aqueous CO_2 concentration in the anoxic tank during SII and SIII mediated by biogas scrubbing supported a desorption of CO_2 from the anoxic tank, resulting in IC REs of 29±12% and 30±6%, respectively.

TN-REs in the anoxic tank increased from 18±8% in SI to 50±6% and 50±7% in SII and SIII, respectively (Figure 2c). This increase in TN removal was likely induced by the enhanced nitrification in the photobioreactor mediated by biogas supplementation, which ultimately promoted $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ reduction in the anoxic tank using the organic matter present in the influent wastewater. In fact, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ represented the main e^- acceptors in SII and SIII, with a contribution to TOC oxidation of 56% and 60%, respectively. The steady state removals of $N\text{-}NH_4^+$ remained low at 6±14%, 9±11% and 2±7% during SI, SII and SIII, respectively. NH_4^+ removal in the anoxic tank was due to biomass assimilation mediated by heterotrophic TOC removal, which remained constant regardless of the operational stage (Figure 2d). $N\text{-}NO_2^-$ concentrations in the anoxic tank under steady state operation were negligible (0.01±0.01 mg/L, 0.03±0.05 and 0.41±0.68 in SI, SII and SIII, respectively).

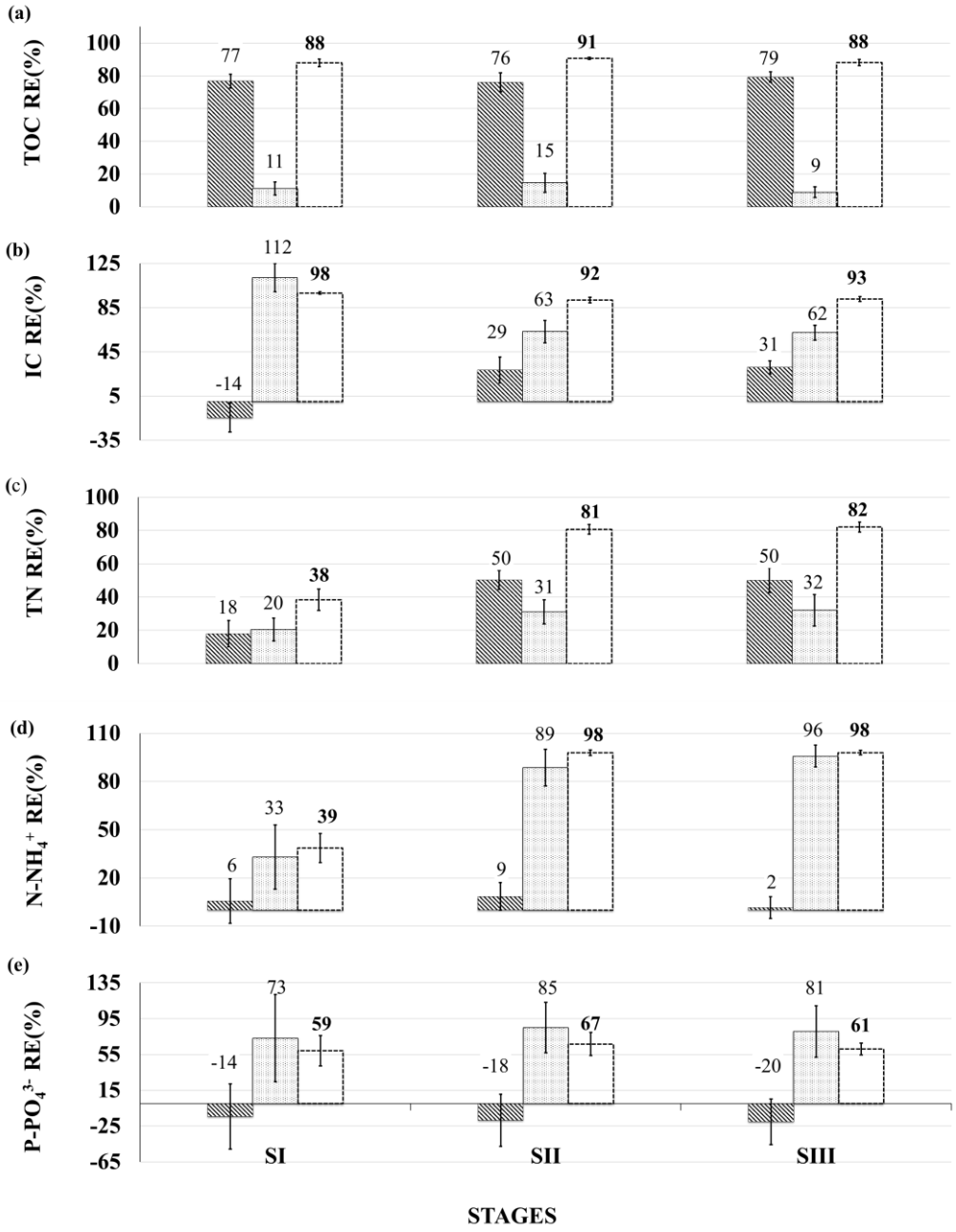


Figure 2. Removal efficiency of (a) TOC, (b) IC, (c) TN, (d) $N\text{-}NH_4^+$ and (e) $P\text{-}PO_4^{3-}$ in the anoxic tank (▨), aerobic photobioreactor (▩) and overall system (▭) during the steady states achieved in the three operational stages evaluated. Vertical bars represent the standard deviation from replicate measurements during steady state operation.

Likewise, $N\text{-}NO_3^-$ concentrations recorded in the anoxic tank in SI, SII and SIII were 0.04±0.03mg/L, 0.14±0.15mg/L and 0.73±1.25mg/L, respectively (Figure 3). These findings confirmed that both NO_2^- and NO_3^- derived from the photobioreactor and settler via the internal and external recirculations were efficiently reduced.

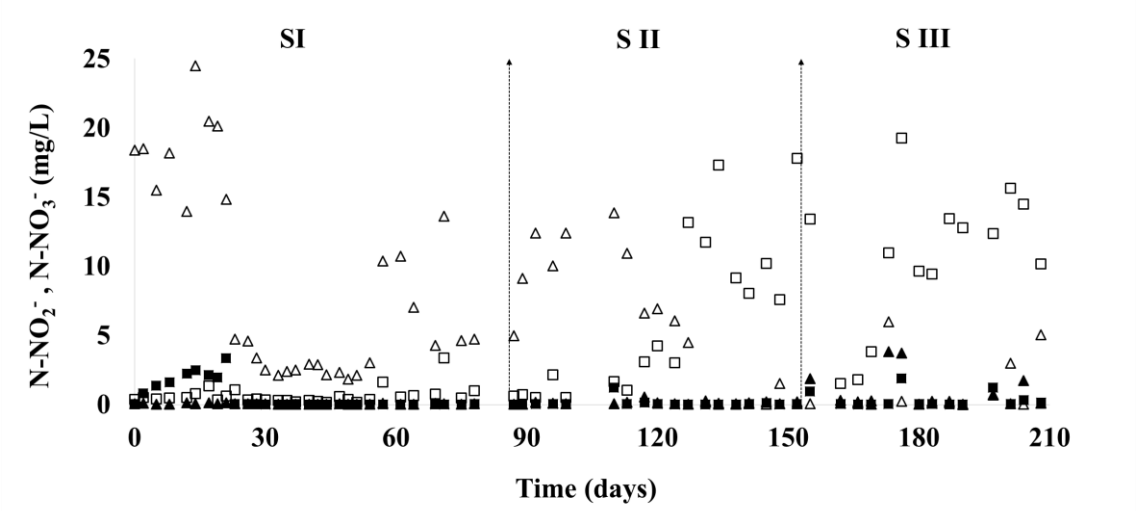


Figure 3. Time course of nitrite (triangles) and nitrate (squares) concentrations in the anoxic tank (black) and photobioreactor (white) during the entire experiment. Vertical dashed lines separate the different operational stages evaluated.

Negative overall $P\text{-}PO_4^{3}$-REs of -17±31% were recorded in the anoxic tank under steady state operation, with $P\text{-}PO_4^{3-}$ removals of -14±36%, -18±29% and -20±25% during SI, SII and SIII, respectively (Figure 2e). These negative $P\text{-}PO_4^{3}$-REs indicated that P was released by the algal-bacterial consortium in the absence of an e^- acceptor (nitrite, nitrate and dissolved oxygen) during SI, SII and SIII, respectively. In this context, recent studies have reported the ability of microalgae to accumulate non-structural $P\text{-}PO_4^{3-}$ under aerobic conditions, which is then released in the absence of e^- acceptor (similarly to phosphate accumulating organisms, PAOs) [30][31]. In addition, the DGGE-sequencing analysis revealed the presence of heterotrophic bacteria with the ability to accumulate energy in the form of polyphosphate under excess of e^- acceptor and use this energy under anoxic conditions with the subsequent release of PO_4^{3-} to the culture medium. Hence, PAOs from the genus *Acinetobacter* (SI, SII and SIII), *Luteolibacter* (SI, SII and SIII), *Thauera* (SII and SIII), *Pseudomonas* (SIII), and *Aeromonas* (SI and SIII) were identified (supplementary materials Table S1) [29][32].

3.2 Carbon and nutrient removal in the photobioreactor

The TOC-REs under steady state operation in the photobioreactor averaged 12±5% regardless of the operational stage as a result of the

efficient removal of organic matter in the anoxic tank (Figure 2a). The consistent concentrations of TOC 19±3 mg/L in the effluent over the entire experiment allowed us to estimate the fraction of non-biodegradable organic matter in the influent RWW to 11%. IC-REs in the photobioreactor under steady state condition accounted for 86±27% as a result of the intensive photosynthetic activity during the illuminated period along the three operational stages in the photobioreactor (Figure 2b). IC was almost completely depleted during the SI (supplementary material Figure S2b). The occurrence of IC limitation during SI supported the addition of biogas in order to supply an additional CO_2 source. Even under CO_2 supplementation, high IC-REs of 63±10% and 62±7% were recorded during SII and SIII, respectively. The enhanced IC availability mediated by biogas upgrading entailed an increase in the concentration of algal-bacterial biomass.

Low TN-REs of 20±7% were recorded under SI steady state, which increased up to 30±7% and 32±9% in SII and SIII, respectively, as a result of the higher biomass production induced by biogas supplementation (Figure 2c). Likewise, while IC limitation mediated low N-NH_4^+-REs (33±20%) during SI, the increase in nitrification activity supported by CO_2 supplementation increased N-NH_4^+-REs up to 89±11% and 96±7% in SII and SIII, respectively (Figure 2d and supplementary material Figure S2d). N-NO_2^- was the dominant form of oxidized nitrogen (N-NO_2^- = 5.6±4.0 mg/L *vs* N-NO_3^- =0.9±0.9 mg/L) during SI (Table 1 and Figure 3). CO_2 supplementation via biogas upgrading promoted nitrification, which resulted in a decrease in N-NO_2^- concentration to 3.1±3.8 mg/L concomitant with an increase in N-NO_3^- concentration up to 8.9±5.5 mg/L in SII [29]. Likewise, an almost complete nitrification was achieved during steady SIII, with N-NO_3^- and N-NO_2^- of 13.0±3.2 mg/L and 1.1±1.8 mg/L of N-NO_2^-, respectively.

The overall steady state P-PO_4^{3-}-REs in the photobioreactor accounted for 80±39%, with values of 73±49%, 85±28% and 81±29% in SI, SII and SIII, respectively (Figure 2e). P assimilation into algal-bacterial biomass was the most likely removal mechanism in the photobioreactor based on the range of pH values recorded during illuminated periods (6.8-9.4) and (6.4-8.1) during the dark periods in SII and SIII.

3.3 Biomass concentration and sludge volumetric index

TSS concentration in the anoxic tank increased from 1519±252 mg TSS/L in SI to 3113±361 mg TSS/L and 2480±309 mg TSS/L during SII and SIII, respectively (Table 1 and Figure 4a). Likewise, biomass concentration in the aerobic tank under steady state operation accounted for 1216±260 mg TSS/L, 2854±324mg TSS/L and 2047±186 mg TSS/L in SI, SII and SIII, respectively.

The fact that TOC removal remained similar along the three operational stages clearly showed that the increase in biomass concentration recorded during SII and SIII was mediated by the enhanced growth of autotrophic microbial communities (microalgae, cyanobacteria and nitrifying bacteria). Finally, effluent TSS concentrations during steady state gradually decreased from 163±83 mg TSS/L in SI, to 81±45 mg TSS/L in SII and 26±12 mg TSS/L in SIII (Figure 4a). The value obtained under steady state in SIII enabled compliance with the European Directive 97/271/CEE [33].

The sludge volumetric index recorded at the end of SI in the anoxic tank and photobioreactor accounted for 95 mL TSS/g and 161 mL TSS/g, respectively (Table 1 and Figure 4b). Surprisingly, the enhanced sedimentation observed during SII, based on the decrease in the effluent TSS concentrations, was not correlated with the SVI in the anoxic tank (128 mL TSS/g) or in the photobioreactor (169 mL TSS/g). These high SVI were likely due to the presence of the filamentous bacteria *Caldilineae* in SI and SII and *Clostridium* in SI, SII and SIII. However, the decrease in SVI recorded during SIII in both the anoxic tank and photobioreactor (80 mL TSS/g and 97 mL TSS/g, respectively) was correlated with low effluent TSS concentrations (Figures 4a and 4b). Overall, SVI of 50 - 100 mL/g in activated sludge plants are considered an indication of a good biomass settling [29]. Low SVI were also reported by Alcántara et al. (2015) in a photobioreactor designed with a continuous biomass recycling. Park et al. (2011) also reported an increase in microalgae settleability by 20% when implementing biomass recycling strategies in HRAPs, which confirmed the key of role of this operational strategy to enhance biomass settling [14][34]. The settling rates of biomass present in the anoxic tank accounted for 1.86 m/h, 1.20 m/h and 1.44 m/h in SI, SII and SIII, respectively. Settling rates of 1.56m/h, 1.02 m/h and 1.47 m/h were recorded for the biomass present in the

photobioreactor in SI, SII and SIII, respectively. An analysis of variance confirmed that the biomass present in the anoxic tank exhibited higher settling rates than the biomass in the photobioreactor in SI and SII. The results here obtained were comparable with those reported by de Godos et al. (2014) and higher than the rates obtained by Alcántara et al. (2015) using a similar AA-ABPh [13][14]. Similarly, 80% of algal biomass present in a HRAP treating domestic WW at 4 days of HRTs exhibited rates higher than 0.4 m/h (Gutierrez et al. 2016) [35].

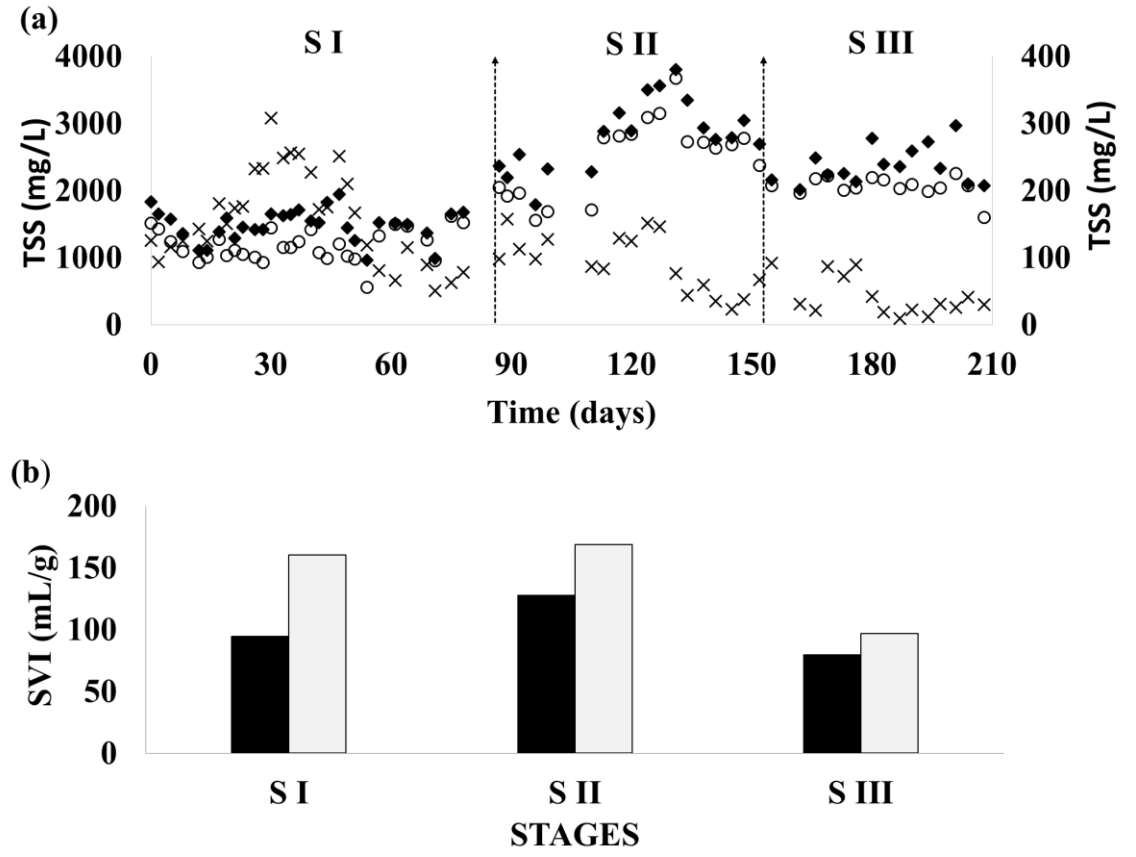


Figure 4. Time course of (**a**) TSS concentration in the anoxic tank (♦) and aerobic tank (○) and effluent (×, secondary axis), and (**b**) **SVI** in the anoxic tank (■) and photobioreactor (□) during the steady states achieved in the three operational stages evaluated. Vertical dashed lines separate the different operational stages.

3.4 Dynamics of microalgae and bacteria population

Morphological characterization of microalgae population structure revealed a gradual dominance of the genus *Scenedesmus*, which accounted for 46 % of total microalgae population in the absence of biogas supply, and for 94-100% when CO_2 was supplemented to the wastewater treatment process (Figure

5). *Desmodesmus spinosus*, *Pseudanabaena sp.*, *Leptolyngbya benthonica* and *Acutodesmus obliquus* represented 38%, 30%, 23% and 8% of the total microalgae population in SI, respectively. In SII, *Leptolyngbya benthonica* and *Pseudanabaena sp* were gradually replaced by *Desmodesmus spinosus* and *Acutodesmus obliquus*, which accounted for 50% and 44% of the total population, respectively. Finally, microalgae population in SIII became dominated by *Desmodesmus spinosus* (76%) and *Scenedesmus tenuispina* (24%). *Scenedesmus* species is commonly found in HRAPs treating domestic WW [36] because of their tolerance to high nitrogen and organic matter concentrations [37][38]. This study suggests that biomass sedimentation and recycling can contribute to the enrichment of monoalgal microalgae species with good settling properties. Previous studies in pilot HRAPs conducted with biomass recycling promoted the dominance of unialgal cultures [34]. In this context, biomass settling and recycling also resulted in the dominance of *Micractinium sp* and *Scenedesmus sp* in HRAPs treating RWW with an external CO_2 supplementation [27].

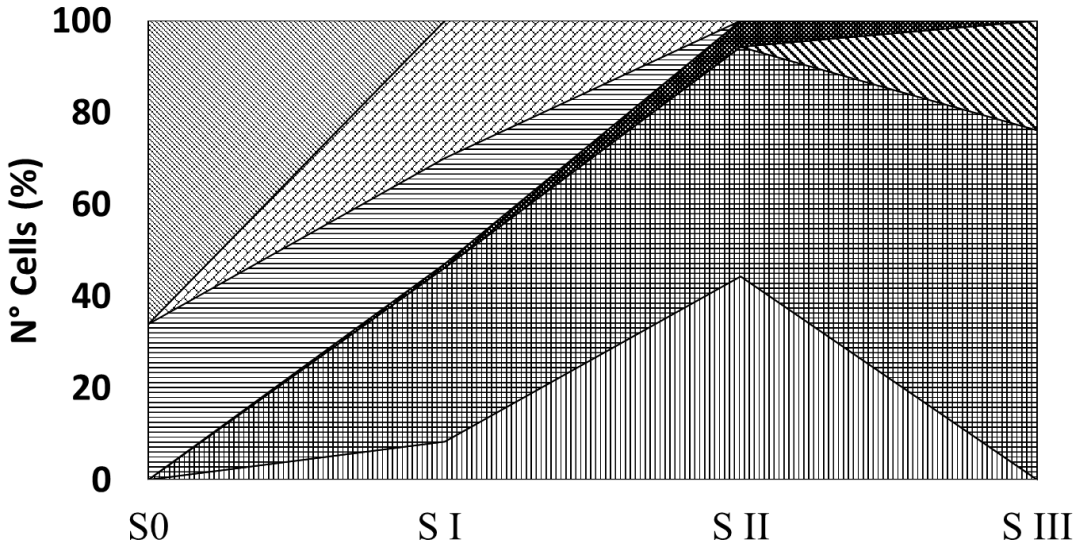


Figure 5. Microalgae population structure in the photobioreactor during the entire operational period: *Chlorella*, *Pseudanabaena sp.*, *Leptolyngbya benthonica*, *Nitzschia palea*, *Scenedesmus tenuispina*, *Desmodesmus spinosus* and *Acutodesmus obliquus*.

DGGE analysis of the bacterial community in the photobioreactor revealed the occurrence of 10 phyla and 33 bands (Figure 6 and supplementary material Table S1). *Proteobacteria*, which are ubiquitous in the environment,

was the dominant phylum with 17 bands of the 33 sequenced. The phylum *Proteobacteria* was the most dominant with 9, 9, 6 and 12 bands detected in the inoculum, SI, SII and SIII, respectively (Figure 6 and supplementary material Table S1)[39]. The analysis also identified the phyla *Acidobacteria*, *Verrucomicrobia*, *Firmicutes* and *Actinobacteria* with two bands each, the phyla *Chloroflexi*, *Cyanobacteria/Chloroplast*, *Gemmatimonadetes*, *Ignavibacteriae*, *Candidatus Saccharibacteria* with one band and 3 unclassified bacteria. Bacteria from the phyla *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Firmicutes* were likely responsible for the degradation of organic matter in both the anoxic and photobioreactor tanks. Bacteria from the above mentioned phyla are typically found in activated sludge WWTP, autotrophic nitrifying and denitrifying bioreactors and HRAPs. More specifically, denitrifying bacteria such as *Pseudomonas* (SIII), *Litorilinea* (SI and SII), *Gp4* (SII) and *Thauera* (SII and SIII) were identified (Figure 6 and supplementary material Table S1). Likewise, nitrifying bacteria belonging to the family *Xhantomonadaceae* (SI, SII and SIII) and genus *Aeromonas* (SI and SIII), *Aquamicrobium* (SI, SII and SIII), *Luteliobacter* (SI, SII and SIII), *Thauera* (SII and SIII) *and Gp4* (SII) were detected as a result on the increased availability of CO_2.

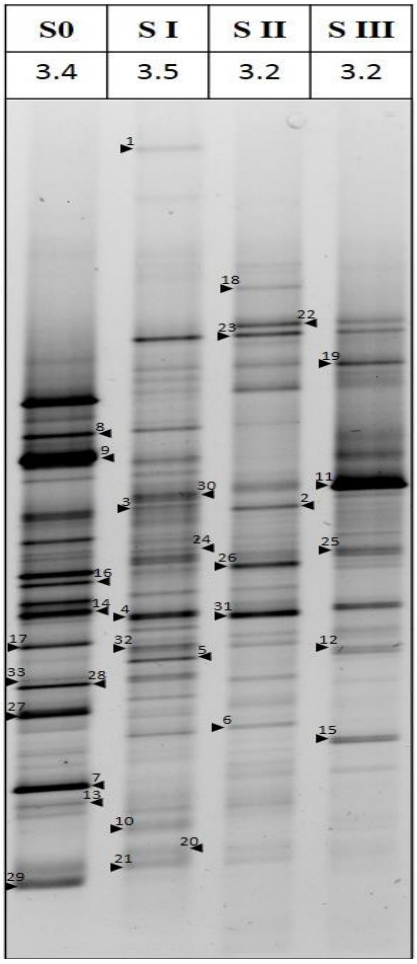


Figure 6. DGGE profile of the bacterial community present in the anoxic-aerobic algal-bacterial photobioreactor in the inoculum (S0), stage I (SI), stage II (SII) and stage III (SIII). Horizontal arrows and numbers indicate the most abundant bacterial communities. The name of the samples and the Shannon-Weiner diversity indexes are also depicted in the upper part of the gel.

The Shannon-Weiner diversity index (H) for the inoculum (S0) and the population established in the different operational stages showed a high bacterial diversity.

The Shannon-Weiner diversity index typically ranges from 1.5 to 3.5, higher H values corresponding to a higher species richness and evenness [16][20]. In this study, H indexes of 3.4, 3.5, 3.2 and 3.2 were estimated in the inoculum and in the microbial populations established during SI, SII and SIII, respectively (Figure 6). HRAPs treating WW typically exhibit H indexes ranging from 3.0 to 3.5, which confirms the high robustness and functionality of the microbiology present in algal-bacterial processes. Both the H index and the DGGE band profile clearly showed that biogas supplementation in SII and SIII stabilized the bacterial community. The analysis of the similarity indexes showed a lower similarity between the inoculum (S0) and the population in SI (25.4%), than the similarity between the populations in SI and II (63.2 %) in SII and SIII (41.30%), which indicated a functional specialization to the host environment during the experiment [40].

3.5 Biogas upgrading

CO_2 supplementation via biogas upgrading was crucial to ensure an efficient nitrification in the photobioreactor and further denitrification in the anoxic tank. CO_2 removal from biogas in the absorption column averaged 92±2% and 93±2 % during steady state II and III, respectively (Figures 7a). No significant absorption of CH_4 was detected in the biogas absorption column here evaluated, likely due to the low solubility of CH_4, which prevented the uncontrolled release of this GHG from the experimental system.

H_2S was completely removed from biogas regardless of the operational stage as a result of its higher solubility compared to CO_2 (Figure 7b). The results obtained here were in agreement with the REs of 80% for CO_2 and 100% for H_2S reported by other authors in HRAPs devoted to biogas upgrading using a similar L/G ratio of ≈ 10 (v/v) [12][41]. While CO_2 supplied was assimilated by nitrifying bacteria and microalgae, H_2S was rapidly oxidized to SO_4^{2-} using the O_2 photosynthetically produced in the photobioreactor. In this context, the removal efficiencies of SO_4^{2-} in the anoxic tank accounted for 92±54% and 16±50% during steady state II and III, respectively (Figure 7c). On the other hand, SO_4^{2-}-REs of -140±58% and -83±60% were recorded in the photobioreactor during steady state II and III, respectively, as a result of SO_4^{2-} production from H_2S oxidation (Figure 7b and 7c). The DGGE sequencing analysis revealed the presence of the H_2S

degrading strain *Pseudomonas frederiksbergensis* NR_117177, which supported the biological oxidation of H_2S in the system (Supplementary material Table S1) [42][43].

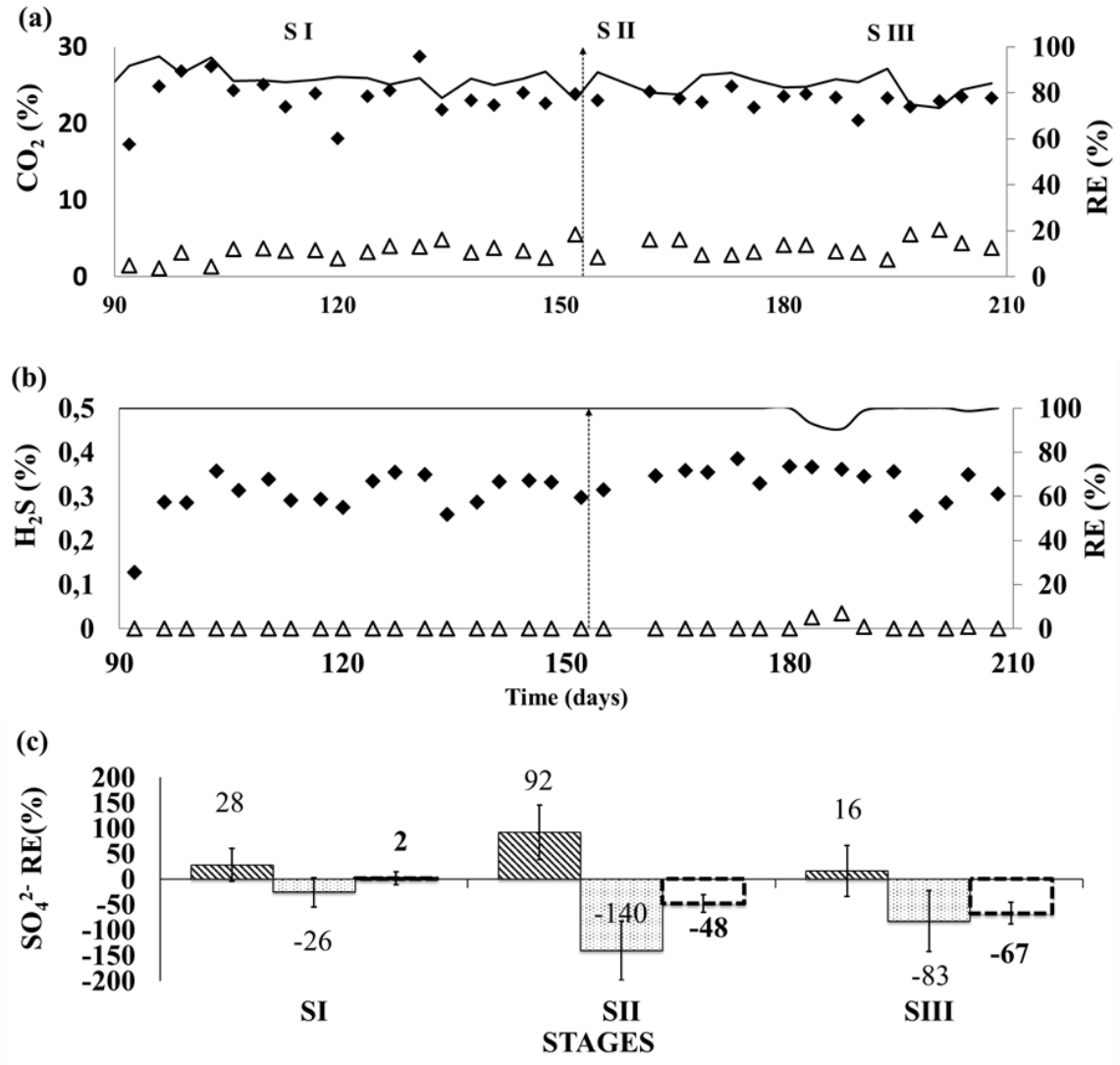


Figure 7. Time course of the inlet (♦) and outlet (Δ) concentrations, and removal efficiency (▬) of CO_2 (a) and H_2S (b), in the absorption column during stage II and III, and (c) removal efficiency of SO_4^{2-} in the anoxic tank (▨), aerobic photobioreactor (▩) and overall system (▭) during the steady states achieved in the three operational stages evaluated. Vertical bars represent the standard deviation from replicate measurements during steady state operation.

4. Conclusion

The novel anoxic-aerobic algal-bacterial photobioreactor coupled with a biogas upgrading unit here evaluated exhibited consistent C, N and P removal efficiencies. CO_2 supplementation from biogas was required to overcome the overall IC limitation recorded in SI, and

supported both an efficient nitrification-denitrification process and an enhanced N and P removal by assimilation during SII and SIII. This innovative process configuration also supported an efficient biogas upgrading, with CO_2 and H_2S removal efficiencies of 85 and 100 %, respectively. Continuous biomass settling and recycling promoted the enrichment of an unialgal culture by the end of the experiment. Finally, DGGE-sequencing analysis confirmed that biogas supplementation promoted the development of nitrifying, denitrifying and H_2S degrading bacteria during SII and SIII.

Acknowledgments

This research was supported by the Spanish Ministry of Economy and Competitiveness and FEDER EU program (CTM2015-70442-R) the Regional Government of Castilla y León (Project VA024U14 and UIC 71) and INIA (RTA2013-00056-C03-02). The financial support of the program ERASMUS MUNDUS: EURICA and Universidad Nacional Autónoma de Nicaragua (UNAN-Managua) are also gratefully acknowledge.

References

[1] FAO, The State of the World's land and water resources for Food and Agriculture. Managing systems at risk, 2011. doi:978-1-84971-326-9.

[2] U.S. Enviromental Protection Agency, CLIMATE CHANGE INDICATOR IN THE UNITED STATES 2016, Fourth edi, 2016. www.epa.gov/climate-indicators.

[3] J. Ruiz, P.D. Álvarez-Díaz, Z. Arbib, C. Garrido-Pérez, J. Barragán, J.A. Perales, Performance of a flat panel reactor in the continuous culture of microalgae in urban wastewater: Prediction from a batch experiment, Bioresour. Technol. 127 (2013) 456–463. doi:10.1016/j.biortech.2012.09.103.

[4] Z. Arbib, J. Ruiz, P. Álvarez-Díaz, C. Garrido-Pérez, J.A. Perales, Capability of different microalgae species for phytoremediation processes: Wastewater tertiary treatment, CO2 bio-fixation and low cost biofuels production, Water Res. 49 (2014) 465–474. doi:10.1016/j.watres.2013.10.036.

[5] A. Ruiz-Martinez, N. Martin Garcia, I. Romero, A. Seco, J. Ferrer, Microalgae cultivation in wastewater: nutrient removal from anaerobic membrane bioreactor effluent., Bioresour. Technol. 126 (2012) 247–253. doi:10.1016/j.biortech.2012.09.022.

[6] R. Muñoz, B. Guieysse, Algal-bacterial processes for the treatment of hazardous contaminants: A review., Water Res. 40 (2006) 2799–2815. doi:10.1016/j.watres.2006.06.011.

[7] M. Wang, H. Yang, S.J. Ergas, P. van der Steen, A novel shortcut nitrogen removal process using an algal-bacterial consortium in a photo-sequencing batch reactor (PSBR), Water Res. 87 (2015) 38–48. doi:10.1016/j.watres.2015.09.016.

[8] L. Christenson, R. Sims, Production and

harvesting of microalgae for wastewater treatment, biofuels, and bioproducts., Biotechnol. Adv. 29 (2011) 686–702. doi:10.1016/j.biotechadv.2011.05.015.

[9] J.B.K. Park, R.J. Craggs, A.N. Shilton, Investigating why recycling gravity harvested algae increases harvestability and productivity in high rate algal ponds., Water Res. 47 (2013) 4904–4917. doi:10.1016/j.watres.2013.05.027.

[10] I. de Godos, V.A. Vargas, S. Blanco, M.C. García González, R. Soto, P.A. García-Encina, et al., A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation, Bioresour. Technol. 101 (2010) 5150–5158. doi:10.1016/j.biortech.2010.02.010.

[11] C. Alcántara, P.A. García-Encina, R. Muñoz, Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process, Chem. Eng. J. 221 (2013) 238–246. doi:10.1016/j.cej.2013.01.100.

[12] M.L. Serejo, E. Posadas, M.A. Boncz, S. Blanco, P. García-Encina, R. Muñoz, Influence of Biogas Flow Rate on Biomass Composition During the Optimization of Biogas Upgrading in Microalgal-Bacterial Processes, Environ. Sci. Technol. (2015) 150212112813001. doi:10.1021/es5056116.

[13] I. de Godos, V.A. Vargas, H.O. Guzmán, R. Soto, B. García, P.A. García, et al., Assessing carbon and nitrogen removal in a novel anoxic-aerobic cyanobacterial-bacterial photobioreactor configuration with enhanced biomass sedimentation., Water Res. 61 (2014) 77–85. doi:10.1016/j.watres.2014.04.050.

[14] C. Alcántara, J.M. Domínguez, D. García, S. Blanco, R. Pérez, P.A. García-Encina, et al., Evaluation of wastewater treatment in a novel anoxic-aerobic algal-bacterial photobioreactor with biomass recycling through carbon and nitrogen mass balances, Bioresour. Technol. 191 (2015) 173–186. doi:10.1016/j.biortech.2015.04.125.

[15] APHA, Standards Methods for the Examination of Water and Wastewater, 21 st, American Public Health Association, American Water Works Association, Water Enviroment Federation, Washington,D.C, 2005. https://www.standardmethods.org/.

[16] J.C. López, G. Quijano, R. Pérez, R. Muñoz, Assessing the influence of CH4 concentration during culture enrichment on the biodegradation kinetics and population structure., J. Environ. Manage. 146 (2014) 116–123. doi:10.1016/j.jenvman.2014.06.026.

[17] A. Sournia, Phytoplankton manual, UNESCO, Paris, 1978. http://unesdoc.unesco.org/images/0003/000307/030788eo.pdf.

[18] U. Nübel, B. Engelen, A. Felske, J. Snaidr, A. Wieshuber, R.I. Amann, et al., Sequence heterogeneities of genes encoding 16S rRNA in Paenibacillus polymyxy detected by temperature gradient gel electrophoresis., J. Bacteriol. 178 (1996) 5636–5643.

[19] K. Roest, H.G.H.J. Heilig, H. Smidt, W.M. de Vos, A.J.M. Stams, A.D.L. Akkermans, Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater., Syst. Appl. Microbiol. 28 (2005) 175–185. doi:10.1016/j.syapm.2004.10.006.

[20] G.M. MacDonald, Space , Time , and Life, John Wiley & Sons, Inc, New York, USA, 2003.

[21] B.G. Häne, K. Jäger, H.G. Drexler, The Pearson product-moment correlation coefficient is better suited for identification of DNA fingerprint profiles than band matching algorithms, Electrophoresis. 14 (1993) 967–972. doi:10.1002/elps.11501401154.

[22] H.-F. Wang, W.-Y. Zhu, W. Yao, J.-X. Liu, DGGE and 16S rDNA sequencing analysis of bacterial communities in colon content and feces of pigs fed whole crop rice., Anaerobe. 13 (2007) 127–133. doi:10.1016/j.anaerobe.2007.03.001.

[23] S. McGinnis, T.L. Madden, BLAST: At the core of a powerful and diverse set of sequence analysis tools, Nucleic Acids Res. 32 (2004) 20–25. doi:10.1093/nar/gkh435.

[24] E. Posadas, M.D.M. Morales, C. Gomez, F.G. Acién, R. Muñoz, Influence of pH and CO2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways, Chem. Eng. J. 265 (2015) 239–248. doi:10.1016/j.cej.2014.12.059.

[25] J. García, R. Mujeriego, M. Hernandez-Mariné, High rate algal pond operating strategies for urban wastewater nitrogen removal, Appl. Phycol. 12 (2000) 331–339. doi:10.1023/a:1008146421368.

[26] J. Mata Álvarez, F. Fdez-Polanco, Ecoeficiencia en la EDAR del Siglo XXI, Santiago de Compostela, España, 2010.

[27] S. Heubeck, R.J. Craggs, A. Shilton, Influence of CO2 scrubbing from biogas on the treatment performance of a high rate algal pond, Water Sci. Technol. 55 (2007) 193–200. doi:10.2166/wst.2007.358.

[28] G. Tchobanoglous, H.D. Stensel, R. Tsuchihashi, F. Burton, Wastewater Engineering Treatment and Resource Recovery, Fifth edit, McGraw-Hill Education, 2014. www.mhhe.com.

[29] B.E. Rittmann, P.L. Mccarty, Environmental Biotechnology:Principles and Applications, 1 st, Tata McGraw-Hill, New Delhi, 2012.

[30] C. Alcántara, C. Fernández, P.A. García-Encina, R. Muñoz, Mixotrophic metabolism of Chlorella sorokiniana and algal-bacterial consortia under extended dark-light periods and nutrient starvation, Appl. Microbiol. Biotechnol. 99 (2015) 2393–2404. doi:10.1007/s00253-014-6125-5.

[31] L.E. De-Bashan, Y. Bashan, Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003), Water Res. 38 (2004) 4222–4246. doi:10.1016/j.watres.2004.07.014.

[32] J. Yoon, Y. Matsuo, K. Adachi, M. Nozawa, S. Matsuda, H. Kasai, et al., Description of Persicirhabdus sediminis gen. nov., sp. nov., Roseibacillus ishigakijimensis gen. nov., sp. nov., Roseibacillus ponti sp. nov., Roseibacillus persicicus sp. nov., Luteolibacter pohnpeiensis gen. nov., sp. nov. and Luteolibacter algae sp. no, Int. J. Syst. Evol. Microbiol. 58 (2008) 998–1007. doi:10.1099/ijs.0.65520-0.

[33] CEC, Consejo de las Comunidaes Europeas. Directiva del Consejo sobre el tratamiento de las aguas residuales urbanas (91/271/CEE), (1991) 40–52.

[34] J.B.K. Park, R.J. Craggs, A.N. Shilton, Recycling algae to improve species control and harvest efficiency from a high rate algal pond., Water Res. 45 (2011) 6637–6649. doi:10.1016/j.watres.2011.09.042.

[35] R. Gutiérrez, I. Ferrer, E. Uggetti, C. Arnabat, H. Salvadó, J. García, Settling velocity distribution of microalgal biomass from urban wastewater treatment high rate algal ponds, Algal Res. 16 (2016) 409–417.doi:10.1016/j.algal.2016.03.037.

[36] I. de Godos, V.A. Vargas, H.O. Guzmán, R. Soto, B. García, P.A. García, et al., Assessing carbon and nitrogen removal in a novel anoxic-aerobic cyanobacterial-bacterial photobioreactor configuration with enhanced biomass sedimentation, Water Res. 61 (2014)

77–85. doi:10.1016/j.watres.2014.04.050.

[37] S. Canovas, B. Picot, C. Casellas, H. Zulkifi, A. Dubois, J. Bontoux, Seasonal development of phytoplankton and zooplankton in a high-rate algal pond, Water Sci. Technol. 33 (1996) 199–206. doi:10.1016/0273-1223(96)00355-1.

[38] C.. Palmer, A composite rating of algae tolerating organic pollution, J. Phicology. 5 (1969) 78–82.

[39] N.-R. Shin, T.W. Whon, J.-W. Bae, Proteobacteria: microbial signature of dysbiosis in gut microbiota., Trends Biotechnol. 33 (2015) 496–503. doi:10.1016/j.tibtech.2015.06.011.

[40] O.H. Diserud, F. Ødegaard, A multiple-site similarity measure, Biol. Lett. 3 (2007) 20–22. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2373804/.

[41] M. Bahr, I. Díaz, A. Dominguez, A. González Sánchez, R. Muñoz, Microalgal-biotechnology as a platform for an integral biogas upgrading and nutrient removal from anaerobic effluents, Environ. Sci. Technol. 48 (2014) 573–581. doi:10.1021/es403596m.

[42] W. Adam, F. Heckel, C.R. Saha-Möller, M. Taupp, J.-M. Meyer, P. Schreier, Opposite enantioselectivities of two phenotypically and genotypically similar strains of Pseudomonas frederiksbergensis in bacterial whole-cell sulfoxidation, Appl. Environ. Microbiol. 71 (2005) 2199–2202. doi:10.1128/AEM.71.4.2199-2202.2005.

[43] S.M. Andersen, K. Johnsen, J. Sørensen, P. Nielsen, C.S. Jacobsen, Pseudomonas frederiksbergersis sp. nov., isolated from soil at a coal gasification site, Int. J. Syst. Evol. Microbiol. 50 (2000) 1957–1964.

SUPPLEMENTARY MATERIAL

Enhanced Carbon, Nitrogen and Phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading.

Dimas García[1], Cynthia Alcántara[1], Saúl Blanco[2], Rebeca Pérez[1], Silvia Bolado[1], Raúl Muñoz[1]*

1-Department of Chemical Engineering and Environmental Technology, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

2- The Institute of the Environment, La Serna 58 - 24007 León, Spain

* Corresponding author: mutora@iq.uva.es

CONTENT

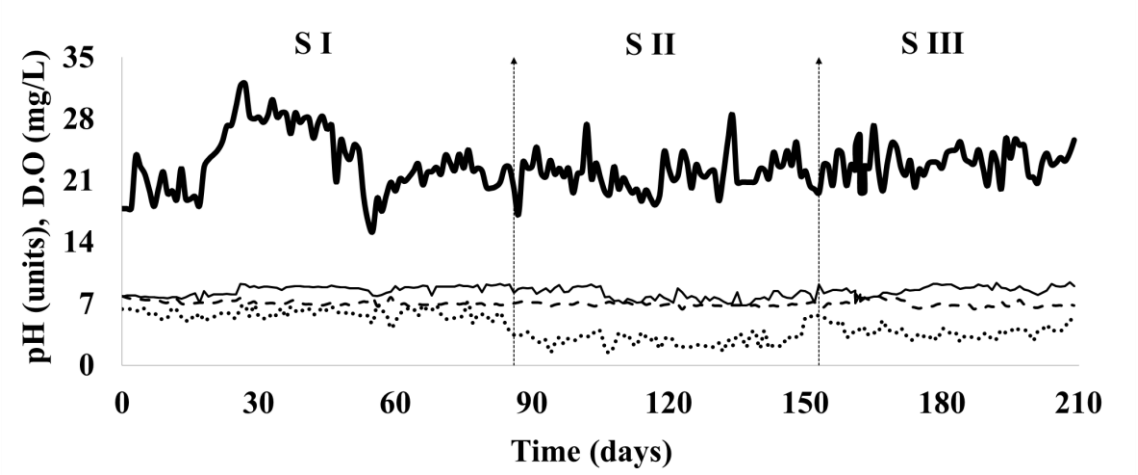


Figure S1. Time course of pH and dissolved oxygen concentration in the photobioreactor during the entire experiment. The thick solid and dotted lines represent the dissolved oxygen concentration during the illuminated and dark periods, respectively. The pH in the illuminated and dark periods is represented by solid and dashed lines, respectively.

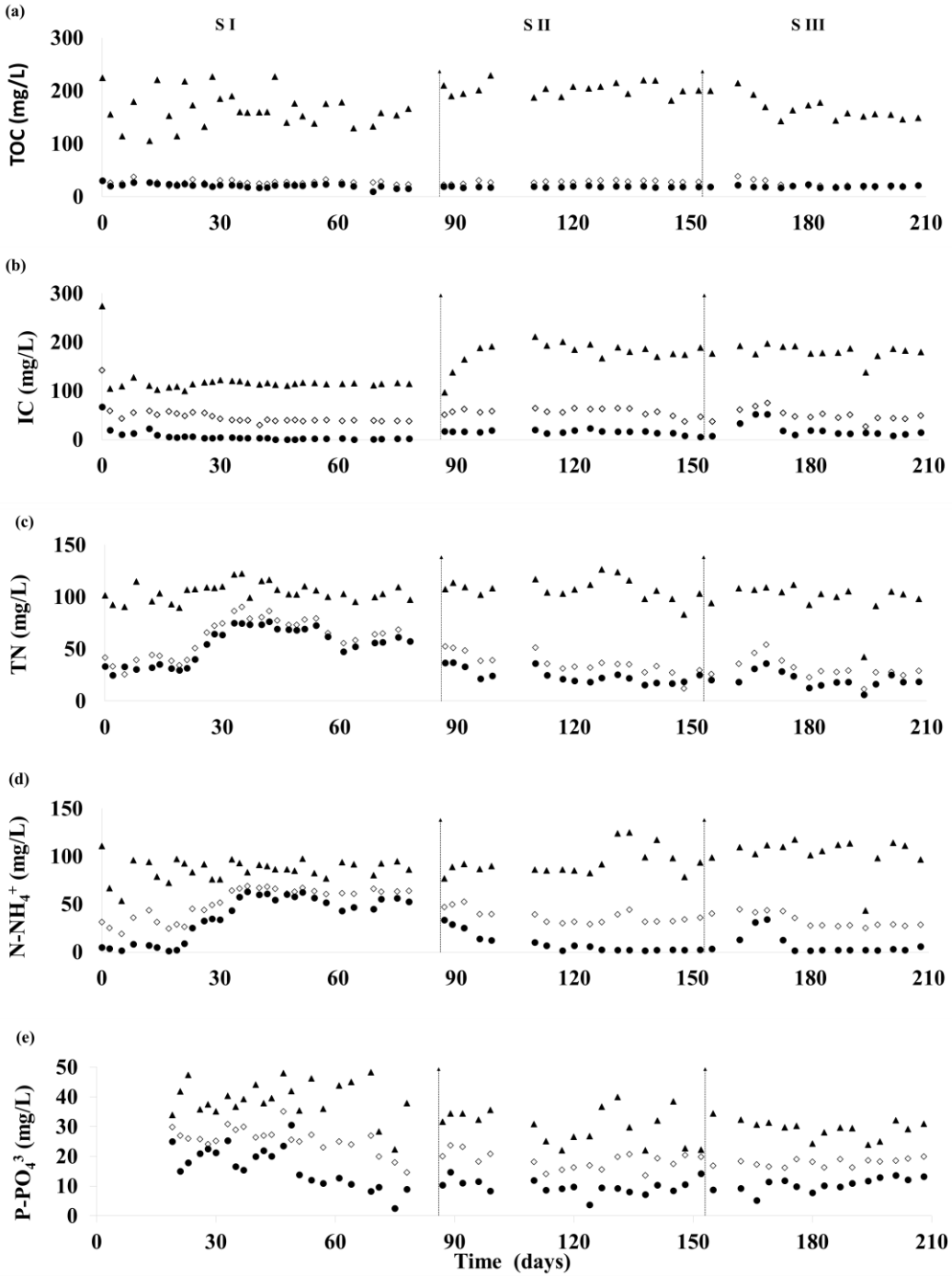


Figure S2. Time course of the concentration of TOC (a), IC (b), $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$ in the RWW (▲), anoxic tank (◊) and photobioreactor (●) during the entire experiment. Vertical dashed lines separate the different operational stages evaluated. IC concentration during SII and SIII accounted for the CO_2 transferred from the biogas upgrading column.

Table S1. RDP classification of the DGGE bands sequenced and corresponding matches (BLASTN) using the NCBI database with indication of the similarity percentages and sources of origin. The presence/absence of each band in each sample together with its intensity are also shown.

Taxonomic placement (50% confidence level)	Band n°	SAMPLES				Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
		S 0	S I	S II	S III			
Phylum Proteobacteria	1		X			Uncultured bacterium (LN562529)	99	Refuse dump Nest 11 layer 3
						Uncultured bacterium (KM290982)	97	Earthworm compost
						Uncultured bacterium (LK392805)	97	Activated sludge treating municipal wastewater
						Uncultured bacterium (KJ940485)	97	Activated sludge
						Uncultured bacterium (FJ660585)	97	Activated sludge
	2	XXX		XX	XX	Uncultured proteobacterium (AY755362)	91	Bioreactor
						Uncultured bacterium (HQ827981)	90	Carpet-like mucilaginous cyanobacterial blooms in a hypereutrophic lake
Class *Gammaproteobacteria*	3		XX		XX	Aeromonas sp. (HG763857)	92	Wheat rhizosphere from saline areas
						Aeromonas sp. (KR189722)	91	Raw water
Order *Xanthomonadales*								
Family *Xanthomonadaceae*	4	XX	XXX		XXX	Uncultured bacterium (GQ480132)	94	Activated sludge from wastewater treatment plant
						Uncultured bacterium (KM290397)	94	Earthworm compost
	5		XXX		XX	Arenimonas donghaensis (NR_043790)	92	Seashore sand
						Uncultured bacterium (HM023338)	92	Surface water around 1m depth
						Uncultured bacterium (JX271974)	92	Activated sludge in lab-scale reactor at 0 cm depth with dissolved oxygen between 0.7 mg/l and 0.9 mg/l
						Uncultured bacterium (HQ640559)	92	Partial nitrifying-ANAMMOX municipal wastewater reactor
						Uncultured bacterium (JX271974)	91	Autotrophic nitrifying bioreactor
	6		XX	XX		Uncultured gamma proteobacterium (KF827303)	91	Biofilm
						Aquimonas voraii (NR_042968)	91	Warm spring
Genus *Pseudofulvimonas*	7	XXX		X	X	Uncultured bacterium (KP797893)	100	Microalgae from HRAP treating diluted vinasse with wastewater treatment plant activated sludge
						Uncultured Xanthomonadaceae (EU305597)	99	Wastewater plant
						Uncultured bacterium (KF911229)	97	Composting process
Order *Aeromonadales*	8		XX			Aeromonas sanarellii (NR_116584)	95	Culture collection
						Aeromonas sp. (KR189870)	95	Raw water
						Aeromonas sp. (KC571192)	95	Activated sludge
						Aeromonas sanarellii (JF920492)	95	Wastewater treatment plant - treated wastewater

Family Aeromonadaceae								
Genus *Aeromonas*	9	XXX				Aeromonas caviae (KJ946377)	99	Wastewater treatment plant influent
						Uncultured bacterium (KP797890)	99	Microalgae from HRAP treating diluted vinasse with wastewater treatment plant activated sludge
						Uncultured Aeromonas sp. (KJ651209)	99	Sludge in MBR for piggery wastewater
	10		XX		X	Uncultured Xanthomonadaceae (KC994700)	98	Microalgae photobioreactor
						Uncultured bacterium (KM290640)	97	Earthworm compost
						Uncultured bacterium (FJ529949)	97	Autotrophic nitrifying bioreactor
						Uncultured Xanthomonadaceae (EF020325)	97	
Order *Pseudomonadales*								
Family *Moraxellaceae*								
Genus *Acinetobacter*	11		XX	XX	XX	Acinetobacter genomo sp. (KR611801)	98	Soil and water ecosystems
						Uncultured bacterium (LK393051)	98	Supernatant of activated treating municipal wastewater
						Acinetobacter harbinensis (KC843488)	98	River
Family *Pseudomonadaceae*								
Genus *Pseudomonas*	12				XX	Pseudomonas sp. (FJ889639)	99	Soil
						Uncultured bacterium (KM292305)	99	Sludge with earthworm
						Pseudomonas sp. (KJ742898)	99	Soil
						Pseudomonas mandelii (KJ726566)	99	River water
						Pseudomonas mandelii (CP005960)	99	Culture collection
						Pseudomonas sp. (KF286228)	99	Shallow aquifer
						Pseudomonas frederiksbergensis (NR_117177)	99	Soil from a gasification site
Class *Betaproteobacteria*								
Order *Rhodocyclales*								
Family *Rhodocyclaceae*	13	XX				Uncultured bacterium (AB488374)	92	Rrice paddy soil
						Uncultured bacterium (JN391796)	92	Biofilm in aerobic tank of hybrid reactor
						Uncultured bacterium (AB608667)	92	Rice paddy soil
						Uncultured bacterium (AB378590)	92	[13C]DNA fraction isolated from paddy soil ammended with nitrate and [13C]succinate
Genus *Thauera*	14	XXX		XX	XX	Uncultured bacterium (HG380609)	96	Wastewater
						Uncultured bacterium (KP641119)	96	Hhigh-rate denitrifying reactor treated with synthetic wastewater
						Thauera sp. (EF205258)	96	Sediment soils
						Thauera phenylacetica (NR_027224)	96	Anaerobic sewage sludge
						Uncultured bacterium (KP136297)	96	Wastewater from high-rate denitrifying reactor

						Uncultured bacterium (KP054189)	96	Nitrification and denitrification reactors
Order *Burkholderiales*	15	XX			XXX	Uncultured bacterium (KM01625)	99	Anaerobic bioreduction of nitrate in hydrogen-based membrane biofilm reactor
						Uncultured bacterium (AB487629)	97	Rice paddy soil
						Uncultured bacterium (AB672290)	97	Rice paddy soil
Class *Alphaproteobacteria*								
Order *Rhodobacterales*								
Family *Rhodobacteraceae*								
Genus *Roseibaca*	16	XXX			XX	Uncultured bacterium (KM823738)	95	River sediment
						Uncultured alpha proteobacterium (EF367342)	94	Mixed biomass developed in SBR lab scale reactors inoculated with activated sludge from a municipal wastewater treatment in Portugal
						Rhodobacteraceae bacterium (AM403177)	93	marine aquaculture
Order *Rhizobiales*								
Family *Phyllobacteriaceae*								
Genus *Aquamicrobium*	17	XXX	XX	XX	XX	Uncultured bacterium (HG380597)	99	Wwastewater
						Uncultured bacterium (FJ167453)	99	Denitrifying bioreactor
						Aquamicrobium aestuarii (NR_108709)	99	Beach sand sample of the Yellow Sea
						Uncultured bacterium (HQ843719)	99	Nitrifying bioreactor
						Uncultured bacterium (JN087889)	99	Nitrifying bioreactor under inorganic carbon limitation
						Aquamicrobium ahrensii (AM884148)	98	Experimental biofilters for the treatment of animal rendering plant waste gases
						Uncultured Aquamicrobium sp. (EU305598)	98	Wastewater plant (WWTP)
Phylum *Acidobacteria*								
Class *Acidobacteria-Gp4*								
Genus *Gp4*	18			XX		Uncultured bacterium (HQ592595)	99	Activated sludge
						Uncultured bacterium (EU244112)	99	River
						Uncultured bacterium (KP054179)	98	Nitrification and denitrification reactors
Genus *Blastocatella*	19		XX	XX	X	Unidentified bacterium (AF097780)	99	Activated sludge
						Unidentified marine bacterium (KC003380)	99	Surface seawater samples from China offshore waters and South China Sea
Phylum *Verrucomicrobia*								
Class *Verrucomicrobiae*								
Order *Verrucomicrobiales*								
Family *Verrucomicrobiaceae*								
Genus *Luteolibacter*	20		XX	X	X	Uncultured bacterium (FJ354094)	97	17th Street Canal, water, greater than 3 micron
						Uncultured bacterium (KM294491)	96	Sludge with earthworm
						Luteolibacter sp. (KC921164)	95	Soil
	21	XX	XX	X	X	Uncultured bacterium (FJ354094)	95	17th Street Canal, water, greater than 3 micron

						Uncultured bacterium (KM294491)	94	Sludge with earthworm
						Uncultured Verrucomicrobium sp. (AY157106)	93	NOX activated sludge system
Phylum *Firmicutes*								
Class *Clostridia*								
Order *Clostridiales*	22			XXX	XX	Uncultured bacterium (KM823668)	92	River sediment
						Clostridium sp. (JF312678)	92	Bioreactor
Family *Peptostreptococcaceae*								
Genus *Clostridium XI*	23		XXX	XXX	XX	Uncultured bacterium (KP797907)	98	Microalgae from HRAP treating diluted vinasse with wastewater treatment plant activated sludge
						Uncultured bacterium (KJ808497)	98	Activated sludge
						Uncultured bacterium (KC551590)	98	Activated sludge
Phylum *Actinobacteria*								
Class *Actinobacteria*								
Subclass *Actinobacteridae*								
Order *Actinomycetales*	24	XX			XX	Uncultured bacterium (JF497778)	99	Activated sludge
						Uncultured bacterium (HQ860566)	97	Stream water
						Uncultured bacterium (KP797903)	96	Microalgae from HRAP treating diluted vinasse with wastewater treatment plant activated sludge
	25		XXX	XX	XXX	Uncultured bacterium (JF497778)	97	Activated sludge
						Uncultured bacterium (HQ860566)	95	Stream water
Phylum *Chloroflexi*								
Class *Caldilineae*								
Order *Caldilineales*								
Family *Caldilineaceae*								
Genus *Litorilinea*	26	XXX	XX	XXX		Uncultured bacterium (HQ014651)	97	Wastewater treatment plant
						Uncultured bacterium (JN391658)	97	Activated sludge in aerobic tank of activated sludge reactor
						Uncultured bacterium (AB576907)	97	Denitrifying PGE pellet samples
Phylum *Cyanobacteria/Chloroplast*								
Class *Cyanobacteria*								
Family *Family IV*								
Genus *GpIV*	27	XXX	X	X	X	Uncultured bacterium (FJ612380)	97	Lake water
						Uncultured bacterium (KF912972)	97	Soil
						Uncultured cyanobacterium (HQ032346)	96	Tailing pound
Phylum *Ignavibacteriae*								
Class *Ignavibacteria*								
Order *Ignavibacteriales*								

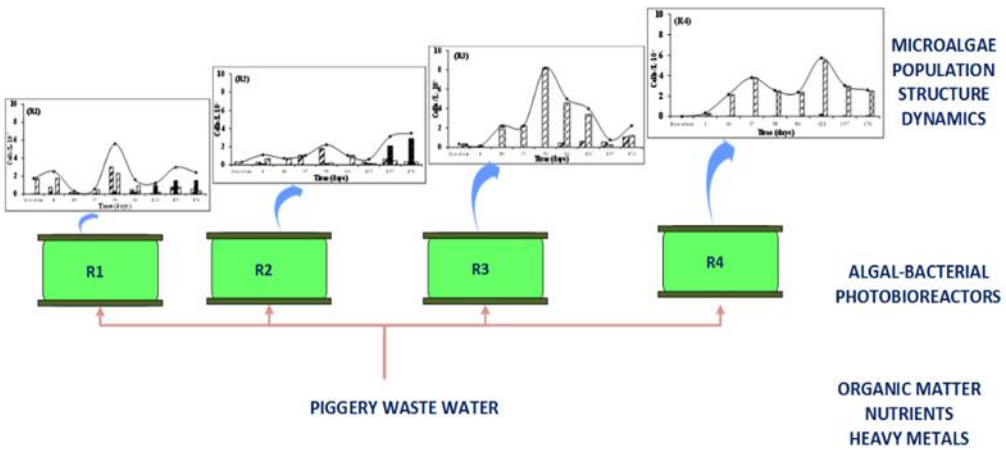
Family *Ignavibacteriaceae*								
Genus *Ignavibacterium*	28	XXX	X	X		Bacterium enrichment (KM210546)	97	Activated sludge from the aerobic tank was enriched with ammonium chloride and sodium sulfate
						Uncultured bacterium (FJ710747)	97	Anaerobic ammonium oxidation reactor
Phylum *Gemmatimonadetes*								
Class *Gemmatimonadetes*								
Order *Gemmatimonadales*								
Family *Gemmatimonadaceae*								
Genus *Gemmatimonas*	29	XXX				Uncultured Gemmatimonadetes (HM022183)	98	Thermomineral spring
						Uncultured Gemmatimonas sp. (EU283561)	98	Lake
						Uncultured bacterium (HM357106)	97	Algal mat
						Uncultured bacterium (GQ441328)	97	Marine microbial mats from a sandy intertidal beach
Phylum *Candidatus Saccharibacteria*								
Genus *Saccharibacteria-genera-incertae-sedis*	30		XX		XX	Uncultured bacterium (JX105655)	93	Ornamental fish aquaria
						Uncultured bacterium (KR306129)	92	Sludge-amended soil with spent mushroom compost day 28
Unclassified Bacteria	31			XXX		Uncultured bacterium (JQ427509)	95	Soil
						Uncultured bacterium (JQ978896)	95	Atrazine-contaminated soil added with inorganic nitrogen and DAT1
						Uncultured bacterium (JQ056068)	94	Soil
	32		XX			Uncultured bacterium (HE647661)	99	CAS wastewater treatment pilot plant
						Environmental 16s rDNA sequence (CU466684)	99	Evry wastewater treatment plant anoxic
						Uncultured bacterium (AB286410)	98	Activated sludge
						Uncultured bacterium (KM340821)	97	Earthworm cast
	33	XX	XX			Uncultured bacterium (KP797908)	91	Microalgae from HRAP treating diluted vinasse with wastewater treatment plant activated sludge

Chapter 2

Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors

García, D., Posadas, E., Blanco, S., Acién, G., García-Encina, P., Bolado, S., Muñoz, R. 2018. Bioresource Technology. 248 (Pt B), 120 -126. doi:10.1016/j.biortech.2017.06.079.

Accepted 14 June 2017



Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors

Dimas García[1,2], Esther Posadas[1], Saúl Blanco[3], Gabriel Acién[4], Pedro García-Encina[1], Silvia Bolado[1], Raúl Muñoz[1]*

[1]Department of Chemical Engineering and Environmental Technology, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

[2]Centro para la Investigación de los Recursos Acuáticos de Nicaragua, CIRA/UNAN-Managua, Apdo. Postal 4598

[3]The Institute of the Environment, La Serna 58 - 24007 León, Spain

[4]Department of Chemical Engineering, University of Almeria, Cañada San Urbano, s/n, 04120 Almeria, Spain

*corresponding author: mutora@iq.uva.es

Abstract

The dynamics of microalgae population during piggery wastewater (PWW) treatment in four open photobioreactors operated at 27 days of hydraulic retention time, and inoculated with *Chlorella* sp. (R1), *Acutodesmus obliquus* (R2), *Oscillatoria* sp. (R3) and in the absence of inoculum (R4), were evaluated for 6 months. In addition, the algal-bacterial biomass concentration, removal of organic matter, nutrients and heavy metals were also assessed. The results revealed a high diversity and rapid variations in the structure of microalgae populations, *Chlorella* sp. being dominant in R4 throughout most of the operational period. Steady state average biomass concentration ranged from 2445-2610 mg/L in R1-R3 to 3265 mg/L in R4. No significant differences were recorded in the removal efficiencies (REs) of total organic carbon (86-87%), inorganic carbon (62-71%), total nitrogen (82-85%) and total phosphorous (90-92%). Finally, Zn-REs accounted for 26% in R3, 37% in R2, and 49% in R1 and R4.

Keywords

Algal-bacterial processes;
Biomass production;
Heavy metal removal;
Microalgae dynamics;
Piggery wastewater treatment.

1. Introduction

The current global energy and climate change crisis has triggered the quest for alternative green energy sources with a low carbon dioxide (CO_2) footprint (González-Fernández et al., 2012a). In this context, microalgae have emerged as a promising renewable energy platform due to their ability to transform sunlight directly into gas biofuels (i.e H_2) or an organic biomass feedstock that can be further bioconverted into multiple liquid and gas biofuels (Richmond, 2004). Thus, microalgal biomass can be anaerobically digested yielding biogas ($CH_4 + CO_2$) and a nutrient rich digestate (Ehimen et al., 2011; González-Fernández et al., 2012b). In addition, while the lipid fraction of microalgae can be transesterified into biodiesel (Vimalarasan et al., 2011), the carbohydrate fraction can be fermented into bioethanol (Naik et al., 2010) or biohydrogen (Chandrasekhar et al., 2015). Microalgae exhibit multiple advantages over conventional energy crops such as high areal productivities (50-100 tn/ha·y), cultivation in non-arable land (preventing competition with food) and high lipid or carbohydrate fractions depending on the cultivation conditions. Likewise, microalgae can be cultivated in fresh, marine or wastewaters (Cheah et al., 2016).

In this context, nutrient-rich wastewaters represent a valuable feedstock to reduce the costs of microalgae and cyanobacteria (from now on referred to as microalgae) cultivation, which will ultimately increase the cost-competitiveness of microalgae-based biofuels (Acién et al., 2016). Algal-bacterial symbiosis can combine a low-cost mass production of biomass with the treatment of wastewater to levels required for discharge into natural water bodies. Indeed, both domestic, industrial and livestock wastewaters have successfully supported microalgae cultivation (Muñoz et al., 2003; Muñoz and Guieysse, 2006). During microalgae-based wastewater treatment, both the organic carbon, nitrogen and phosphorous present in the residual effluent are assimilated into algal-bacterial biomass. Heavy metals and pathogens are also efficiently removed during microalgae growth as a result of adsorption and pH-mediated mechanisms. Despite microalgae cultivation in wastewater entails significant economic and environmental advantages over axenic mass production of microalgae in mineral salt media, controversy still exists in literature about the possibility of maintaining monoalgal

cultures with a constant biomass composition during microalgae-based wastewater treatment. This is central to the development of microalgae-based biorefineries for biofuel production, whose viability depends on the supply of a biomass with a consistent year-round composition and characteristics. Hence, while most studies conducted under laboratory or outdoors conditions focused on the removal of key pollutants present in wastewater, little attention has been paid to the monitoring of the dynamics of microalgae population.

Pig production is a key economic sector in many countries in Europe, accounting for 148.7 million pigs heads and 44.3% of the total European livestock (EU, 2015; MAGRAMA, 2015) in 2015. European pig farming generates 217-434 million m^3/y (4-8 L/day/pig) of piggery wastewater containing high concentrations of organic matter and nutrients (De Godos et al., 2009). The estimated average organic matter and nutrient load present in EU piggery wastewaters in 2015 amounted to 8.923.000 tn chemical oxygen demand (COD)/y, 890.000 tn nitrogen (N)/y and 223.000 tn phosphorous (P)/y (EU, 2016). In addition, piggery wastewater can contain high concentrations of heavy metals such as Zinc and Copper, typically used as growth promoters in swine nutrition (Abe et al., 2012; De la Torre et al., 2000).

The experimental work herein conducted evaluated the dynamics of microalgae population during piggery wastewater treatment in four open continuous photobioreactors inoculated with two green microalgae species, a cyanophyta, and without inoculum. In addition, the influence of the microalgae inoculum on the steady state organic matter, nutrient and heavy metal removal was assessed.

2. Materials and methods

2.1 Microalgae

Chlorella minutissima Fott and Nováková was obtained from an indoor high rate algal pond (HRAP) treating centrate at the Dept. of Chemical Engineering and Environmental Technology from Valladolid University (Spain). *Acutodesmus obliquus* and *Oscillatoria sp* were kindly provided by the Department of Chemical Engineering from Almeria University (Spain).

2.2 Piggery wastewater

Fresh centrifuged piggery wastewater (PWW) was collected at a nearby farm at Cantalejo (Spain) and stored at 4°C. The

average composition of the piggery wastewater diluted at 15% was: 1340±34 mg/L of total suspended solids (TSS), 1375±121 mg/L of total organic carbon (TOC), 314±55 mg/L of inorganic carbon (IC), 393±26 mg/L of total nitrogen (TN), 9.4±0.4 mg/L of total phosphorus (TP) and 0.7±0.2 mg/L of zinc (Zn). Nitrate (NO_3^-), nitrite (NO_2^-), copper (Cu) and arsenic (As) concentrations remained below detection limit (Table 1).

2.3 Experimental set-up

The experimental set-up consisted of four 15.8 cm deep 3 L open photobioreactors illuminated at 2800 µmol/m^2·s for 12 hours a day (08h00 to 20h00) by LED lamps arranged in a horizontal configuration 20 cm above the photobioreactor surface (Figure1). The photobioreactors were immersed in a water bath to prevent the high temperatures imposed by the LEDs irradiation. Immersion water pumps were used to mix the algal-bacterial cultivation broth in the reactors. The photobioreactors were fed with piggery wastewater diluted at 15% using an auto control 205U7CA multi-channel cassette pump (Watson-Marlow, UK). The pH in the cultivation broth was automatically maintained at 8.0 via CO_2 addition (CARBUROS METALICOS-Barcelona, Spain) using a Crison multimeter M44 control unit (Crison Instruments, Spain).

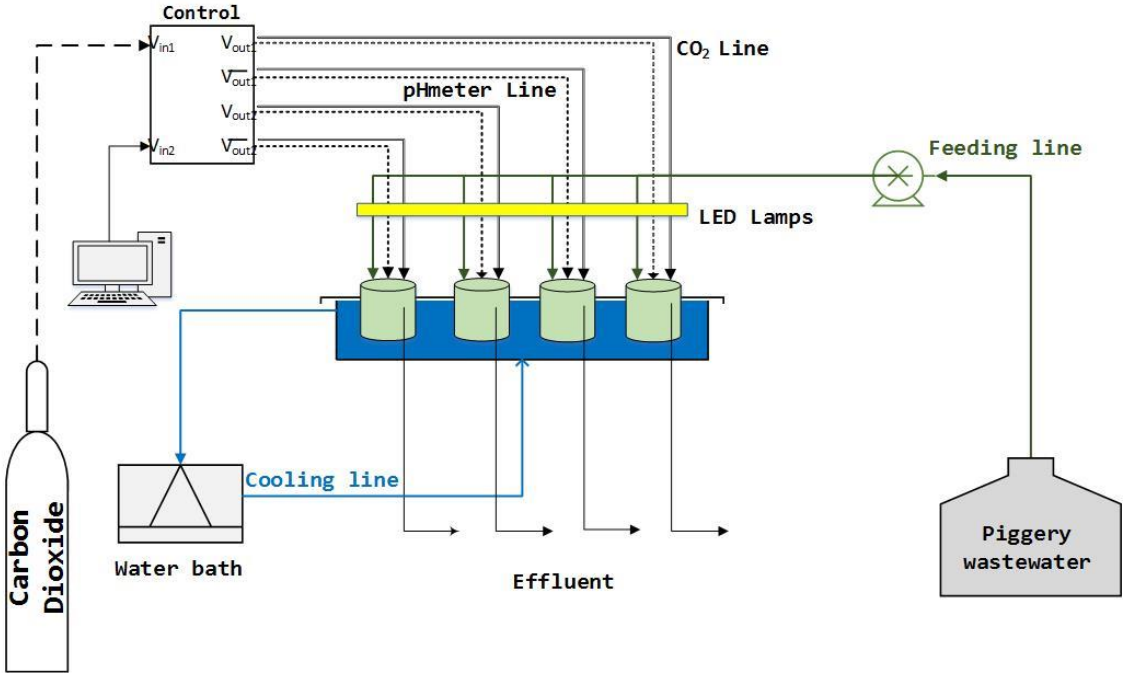


Figure 1. Schematic diagram of the algal-bacterial photobioreactor set-up using carbon dioxide supplementation for pH control.

2.4 Experimental design

Photobioreactors 1, 2 and 3 (namely R1, R2 and R3, respectively) were inoculated with *Chlorella minutissima* Fott and Nováková, *Acutodesmus obliquus* and *Oscillatoria* sp., respectively, at an initial TSS concentration of 220 mg/L (corresponding to initial cell concentrations of 1.750, 0.295 and $0.332 \cdot 10^9$ cells/L, respectively). Photobioreactor 4 (R4) was not inoculated and served as control. The photobioreactors, which were initially filled with tap water, were operated at a hydraulic retention time (HRT) of ≈ 27 days (estimated based on the influent PWW) for 176 days. Photobioreactors effluents overflowed separately as a function of the evaporation rates. Liquid samples of 30 mL were weekly drawn from the influent PWW and effluent of R1, R2, R3 and R4 to determine the concentrations of TOC, IC, TN, NO_2^-, NO_3^-, TP and TSS. Effluent samples were filtered through 1 µm glass fiber filters prior analysis. Likewise, the microalgae population structure in R1, R2, R3 and R4 was weekly assessed from biomass samples preserved with lugol acid at 5% and formaldehyde at 10%, and stored at 4 ºC prior to analysis (only 8 samples from each photobioreactor were analyzed). The dissolved oxygen and temperature of the cultivation broths were measured twice per day, while the influent and effluent flowrates were daily recorded in all photobioreactors to monitor water evaporation losses. Finally, the C, N and P content of the algal bacterial biomass was measured under steady state at the end of the experiment.

The C, N and P removal efficiencies (RE) were calculated according to Eq. (1):

$$RE(\%) = \frac{(C_{feed} \times Q_{feed}) - (C_{eff} \times Q_{eff})}{C_{feed} \times Q_{feed}} \times 100 \quad (1)$$

where C_{feed} and C_{eff} represent the dissolved concentrations of TOC, IC, TN, TP and Zn in the PWW and photobioreactors effluents, respectively, while Q_{feed} and Q_{eff} represent the PWW and effluent flow rates. The process was considered under steady state when the TSS concentrations in the photobioreactors remained stable for at least four consecutive samplings (~ 1 month). The results were here provided as the average ± standard deviation from duplicate measurements along one month of steady state (days 150-176).

2.5 Analytical procedures

A Crison M44 multimeter and a Crison PH 28 meter were used for the on-line measurement of the pH. Dissolved oxygen (DO) and temperature (T) were recorded using an OXI 330*i* oximeter (WTW, Germany). A LI-250A light meter (LI-COR Biosciences, Germany) was used to measure the light intensity as photosynthetically active radiation (PAR). TOC, IC and TN concentrations were determined using a TOC-V CSH analyzer equipped with a TNM-1 module (Shimadzu, Japan). Nitrate and nitrite were analyzed by high performance liquid chromatography-ion conductivity (HPLC-IC) in a Waters 515 HPLC pump coupled with a Waters 432 ionic conductivity detector and equipped with an IC-Pak Anion HC (150 mm × 4.6 mm) column. TSS and TP concentrations were determined according to Standard Methods (APHA, 2005). The analysis of the C, N and P content in the algal-bacterial biomass was carried out using a LECO CHNS-932 elemental analyzer with pre-dried and grinded algal-bacterial biomass. The concentration of Zn, Cu and As was determined using a 725-ICP Optical Emission Spectrophotometer (Agilent, USA) at 213.62. The identification and quantification of microalgae were conducted by microscopic examination (OLYMPUS IX70, USA) according to Phytoplankton Manual (Sournia, 1978).

Table 1. Physical/chemical characterization of the diluted swine manure and cultivation broth in the photobioreactors at steady state.

Parameter	PWW	R1	R2	R3	R4
Evaporation (%)	n.a	60±6	60±7	60±6	60±8
Temperature (ºC)	n.a	30±2	30±2	30±2	30±2
Dissolved Oxygen (mg/L)	n.a	0.8	1.1	1.3	0.9
TOC (mg/L)	1375±121	459±31	452±31	482±27	490±37
IC (mg/L)	314±55	285±14	242±34	227±33	294±27
TN (mg/L)	393±26	174±11	166±15	165±12	149±10
Nitrite (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Nitrate (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
TP (mg/L)	9.4±0.4	2.4±0.3	2.1±0.2	1.9±0.5	1.8±0.3
Zinc (mg/L)	0.7±0.2	0.9±0.2	1.1±0.1	1.3±0.3	0.9±0.3
Copper (mg/L)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
Arsenic (mg/L)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
TSS (mg/L)	1340±34	2610±191	2569±69	2445±222	3265±133
n.a : Not applicable					

3. Results and Discussion

3.1 Dynamics of microalgae population

Chlorella sp., the inoculated microalgae species in R1, was detected throughout most of the experimental period in this photobioreactor and dominant at days 37 and 86 (at concentrations of $0.5 \cdot 10^9$ and $0.9 \cdot 10^9$ cells/L, respectively). *Acutodesmus obliquus* was also identified in R1 and became the dominant species by day 58. Finally, *Aphanothece* sp. was detected for the first time by day 58 and was dominant from day 122 to the end of the operation of R1 (Figure 2a). Similarly, the inoculated microalga species in R2 (*Acutodesmus obliquus)* was identified along the entire photobioreactor operation, with a significant dominance by days 37, 58 and 122 at cell concentrations of $1.3 \cdot 10^9$, $1.8 \cdot 10^9$ and $0.3 \cdot 10^9$ cells/L, respectively. *Chlorella* sp. was identified in R2 from the first operational days and remained at similar cell concentrations throughout the entire experiment (from $0.3 \cdot 10^9$ to $0.7 \cdot 10^9$ cells/L). Finally, *Aphanothece* sp. became dominant in R2 by the end of operation, with final cell concentrations of $2.9 \cdot 10^9$ cells/L (Figure 2b). *Oscillatoria* sp. was replaced by *Chlorella* sp. and *Acutodesmus obliquus* in R3 from the first operational days (after the inoculation a change in color from green to red was noticed), *Chlorella* sp. being the dominant species throughout the entire operation with a maximum concentration of $8.2 \cdot 10^9$ cells/L by day 58 (Figure 2c). The higher pollution-tolerance of *Chlorella* sp. to PWW, combined with the high temperature and irradiations prevailing in this study, could have caused this rapid replacement of *Oscillatoria* sp (Talbot et al., 1991). Despite R4 was not inoculated, *Chlorella* sp. and *Aphanothece* sp. were present in the photobioreactor from the first days, *Chlorella* sp. being the dominant species along the 6 months of experiment. The gradual increase in number of cells of *Aphanothece* sp. in R1, R2 and R4 suggest the influence of the characteristics of the PWW on microalgae population (Figure 2).

The higher dominance of *Chlorella* sp. in the four photobioreactors confirmed the high tolerance of this green microalgae to the pollutants and concentrations typically present in PWW (Kim et al., 2016; Kuo et al., 2015; Yuan et al., 2013). Indeed, the high abundance of *Acutodesmus obliquus* and *Chlorella* sp. (both belonging to the Chlorophyta phylum) along the experimental period

in R1, R2 and R3 matched the microalgae pollution-tolerance classification reported by Palmer et al. (1969), who ranked *Scenedesmus* and *Chlorella* 4th and 5th, respectively. It can be hypothesized that organic pollution exhibited a higher influence on microalgae population structure than other environmental parameters such as water hardness, light intensity, pH, DO or temperature (Palmer, 1969). On the other hand, *Aphanothece* sp., which was not previously classified as a pollution tolerant microalga, was mainly identified at the end of experiment in R1 and R2 (Palmer, 1969). However, *Aphanothece microscopica nägeli* and *Aphanothece Clathrata* successfully supported the removal of organic matter and nitrogen from parboiled rice wastewater (REs of 83.4 and 72.7% for COD and N-TKN, respectively) in a 4.5 L tubular photobioreactor operated batchwise for 24 hours (Queiroz et al., 2007). Likewise, Bastos et al. (2014) reported COD and N-TKN REs of 97 and 78%, respectively, in a 4L batch tubular reactor treating parboiled rice wastewater for 24 hours.

The lack of monoalgal cultures in the four photobioreactors throughout the experimental period and the rapid variations in microalgae population structure here recorded (mainly in R1 and R2) revealed the difficulty to maintain monoalgal cultures during the treatment of PWW in open systems (Posadas et al., 2015). In this context, a lower microalgae diversity was observed at higher biomass concentrations, which was in agreement with Park et al. (2011). In addition, the current morphological microalgae characterization revealed that the inoculation of a photobioreactor during PWW treatment with a specific microalga does not guarantee its long-term dominance (Serejo et al., 2015). Finally, it should be stressed that the different microalgae cells concentration in the inoculum of the photobioreactors (1.750, 0.295 and $0.332 \cdot 10^9$ cells/L for R1, R2 and R3, respectively) only affected the time required to reach steady state and the initial treatment performance, but it did not modify the conclusions here obtained since the performance of the systems was analyzed at constant under steady state.

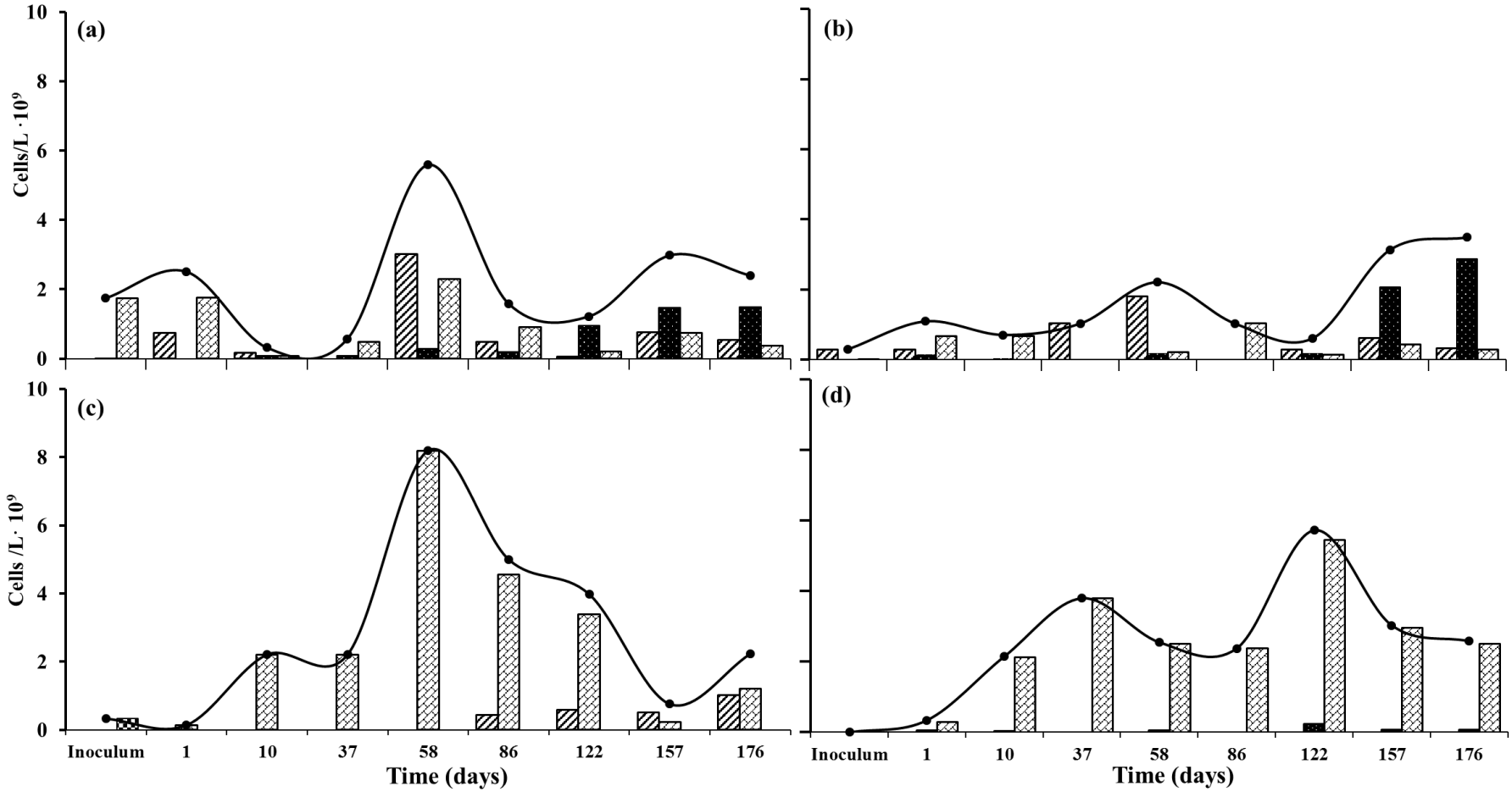


Figure 2. Time course of microalgae population structure in (**a**) R1, (**b**) R2, (**c**) R3 and (**d**) R4. *Acutodesmus obliquus* (▨), *Aphanothece* sp. (■), *Chlorella* sp. (▩), *Oscillatoria* sp. (▩) and total number of microalgae cells (●).

3.2 Biomass concentration and productivity

Biomass concentration in R1, R2 and R3 increased from 220 mg TSS/L to 530, 680, and 660 mg TSS/L, respectively, during the first 38 days. A moderate increase from 0 to 200 mg TSS/L was also recorded in R4 (Figure 3). A significant biomass concentration increase occurred in R1, R2 and R3 from the day 38 to 93, when TSS concentrations of 2440, 2140 and 2500 mg TSS/L, respectively, were measured. However, a lower biomass growth rate was observed during this period in R4, where concentrations up to 1200 mg TSS/L were recorded (Figure 3). Biomass concentration in R2 and R3 remained constant from day 93 onwards at average concentrations of 2569±69 and 2445±222 mg TSS/L, respectively. Biomass concentration in R1 fluctuated from day 93 to 150, to finally stabilize at 2610±191 mg TSS/L, which was similar to the concentrations reached in R2 and R3 (Figure 3). On the other hand, biomass concentration exponentially increased in R4 from day 93, to reach average value of 3265±133 mg TSS/L by the end of the experiment. Surprisingly, the highest algal-bacterial biomass concentration under steady state was achieved in the non-inoculated photobioreactor despite its longer lag phase. Likewise, the highest TOC, IC, TN, TP and Zn REs (below discussed) were obtained in R4, which highlighted the higher robustness of native microalgae species acclimated to the environmental and operational conditions prevailing during PWW treatment (Figures 2 and 3, Table 1) (Olguín et al., 2013). In addition, the results clearly showed a similar biomass growth pattern in the photobioreactors inoculated with a specific photosynthetic microorganisms in comparison with the control unit R4.

The high biomass concentrations here recorded were supported by the high carbon and nutrients concentrations in the diluted PWW and by the high water evaporation rates in the systems, which accounted for 60 % of the influent PWW in all photobioreactors as noticed by Guieysse et al. (2013) (Table 1). Hence, biomass productivities under steady state averaged 6.2±0.5, 6.1±0.2, 5.8±0.6 and 7.8±0.3 $g/m^2 \cdot d$ in R1, R2, R3 and R4, respectively. These productivities were comparable to those obtained during the treatment of secondary domestic wastewater treatment in pilot raceways at high HRT in Almeria (Spain), and were likely limited by the long HRT needed to ensure satisfactory organic

matter and nutrients removals (Posadas et al., 2015).

Finally, the comparison between the evolution of the total number of microalgae cells in the cultures and the TSS concentrations (Figures 2 and 3) showed no direct correlation as a result of the dominant role of bacteria in the process, which itself was influenced by the high biodegradable organic matter load. In this regard, an accurate empirical determination of the individual bacteria and microalgae populations would bring valuable insights about the mechanisms underlying organic matter and nutrient removal during PWW treatment.

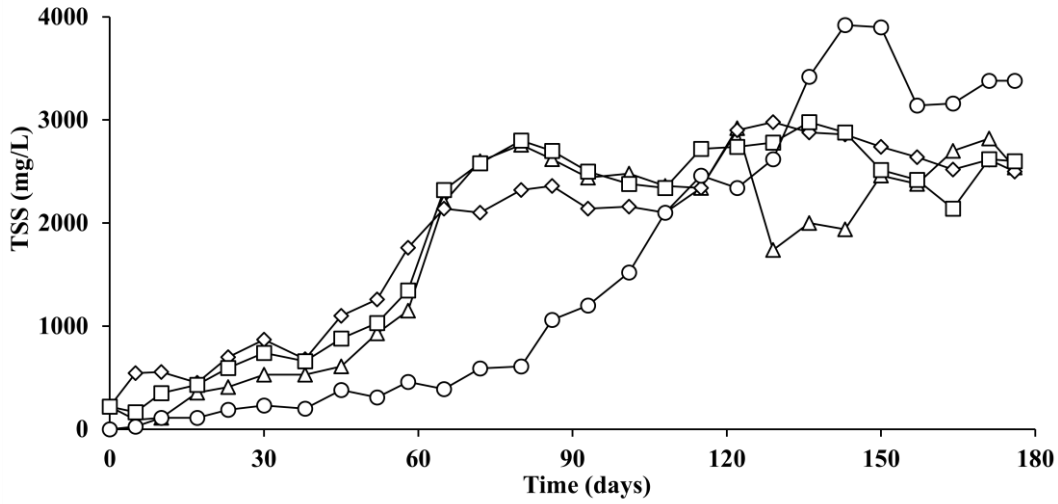


Figure 3. Time course of TSS concentration in R1 (Δ), R2 (◊), R3 (□) and R4 (○)

.

3.3 Carbon and nutrient removal

A comparable bioremediation performance in terms of TOC, IC, TN and TP removal was recorded regardless of the microalgae inoculated in the photobioreactor (Figure 4 and Table 1). In this context, the dominant microalgae species prevailing in the photobioreactor did not influence process performance. In this particular study, the high light irradiances and the optimum temperature for microbial activity supported an effective PWW treatment. Thus, despite the low DO concentrations in the cultivation broth (≤1.3 mg/L), TOC-REs accounted for 86±1, 87±5, 86±1 and 86±1 % in R1, R2, R3 and R4, respectively, which resulted in average TOC concentrations in the effluent at the end of the operational period of 459±31, 452±31, 482±27 and 490±37 mg/L, respectively (Figure 4 and Table 1). Please note that the high water evaporation rates typically encountered

in open photobioreactors resulted in moderately high effluent TOC concentration despite the high removal efficiencies achieved. The results herein obtained confirmed the consistent removal of organic matter from PWW by algal-bacterial processes and were in agreement with the study conducted by De Godos et al. (2009), who reported COD removal efficiencies of 76±11% in a 464 L high rate algal ponds (HRAP) during the treatment of 20 and 10 folds diluted PWW. Similarly, IC-REs of 63±3, 69±4, 71±4 and 62±3 % were recorded at the end of the process in R1, R2, R3 and R4, respectively, which resulted in IC concentrations in the cultivation broth of the photobioreactors of 285±14, 242±34, 227±33 and 294±27 mg/L, respectively (Figure 4 and Table 1). These high IC-REs were promoted by the intensive photosynthetic activity during the illuminated period over the 176 days of operation. However, carbon removal by stripping (prior mineralization of the organic carbon to CO_2) was the main mechanism accounting for carbon fate, since only 37, 38, 36 and 48 % of the total carbon removed was recovered in the harvested biomass in R1, R2, R3 and R4, respectively, under steady state conditions. This estimation was based on the carbon content of the biomass under steady state (as described below) and did not account for the CO_2 input for pH control.

TN-REs of 82±1, 83±3, 83±1 and 85±1 % were recorded under steady state in R1, R2, R3 and R4, respectively, which resulted in TN concentrations in the photobioreactor effluent of 174±11, 166±15, 165±12 and 149±10 mg/L, respectively (Figure 4 and Table 1). These high TN effluent concentrations in spite of the effective nitrogen removal efficiencies were due to the high evaporation rates in the photobioreactors. The TN-REs here recorded were similar to those reported by De Godos et al. (2009), who measured average total kjeldahl nitrogen (TKN) removals of 86±6% during PWW treatment in an open HRAP, and higher than the TN-REs of 63% obtained during the treatment of PWW under laboratory conditions in a 500 ml conical flasks incubated on a rotatory shaker at 27 ºC and 150 rpm under continuous illumination (Abou-Shanab et al., 2013). Likewise, Posadas et al., (2017) operated an outdoors HRAP supporting TN-REs of 80-86% during the treatment of centrate. Nitrogen removal by stripping was the main mechanism in our study, since only 26, 26, 23 and 31 % of the total nitrogen removed was recovered in

the harvested biomass in R1, R2, R3 and R4, respectively.

On the other hand, steady state TP-REs of 90±2 , 91±1, 92±2 and 92±2 % were recorded in R1, R2, R3 and R4, respectively, which supported effluent TP concentrations of 2.4±0.3, 2.1±0.2, 1.9±0.5 and 1.8±0.3 mg/L, respectively (Figure 4, Table1). The TP-REs as (P-PO_4^{3-}) herein obtained were similar to those reported by Posadas et al., (2017) during the treatment of centrate in an outdoors HRAP (84 - 92%). Likewise, the TP-REs reported were in agreement with Franchino et al. (2016), who recorded phosphate REs > 90% during the treatment of 5 and 10 times diluted digestate in 250 ml flasks. Phosphorous assimilation into algal-bacterial biomass was the main removal mechanism in the photobioreactors based on the moderate pH values prevailing in the photobioreactors during the entire experiment (pH=8), which did not support a significant phosphate precipitation (García et al., 2017). Thus, a phosphorus mass balance revealed that 93, 93, 96 and 100 % of the total phosphorus removed was recovered in the harvested biomass for R1, R2, R3 and R4, respectively.

Overall, it is worth noting that a similar macroscopic bioremediation performance was recorded in the photobioreactors in spite of the different microalgae population structures under steady state (and during most of the experiment period), which suggest that bacteria played a dominant role during the treatment of high strength wastewaters such as piggery effluents.

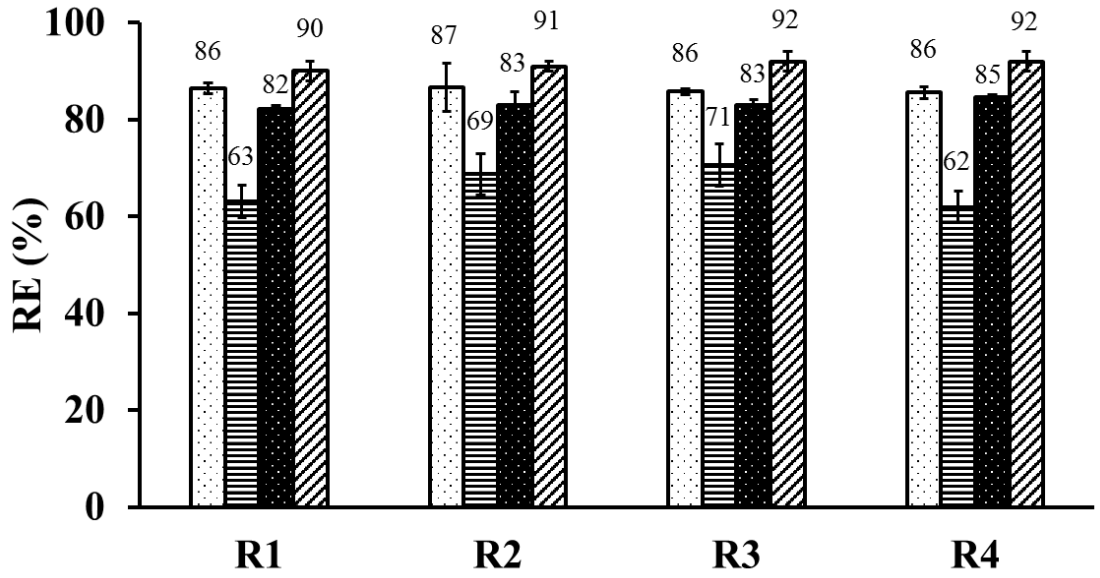


Figure 4. Average removal efficiencies of TOC (▭), IC (▤), TN (■) and TP (▨) under steady state. Vertical bars represent the standard deviation from replicate measurements during steady state operation.

Finally, comparable carbon, nitrogen and phosphorus contents were measured in the harvested biomass under steady state regardless of the initial inoculum, with average values of 50±1, 7.8±0.3 and 0.75±0.06 % for C, N and P, respectively (Figure 5). These elemental biomass compositions were similar to those reported by Posadas et al. (2013) during domestic wastewater treatment in a 15 L algal-bacterial biofilm photobioreactor (42±2, 7±1 and 1.3±0.3 % for C, N and P, respectively), despite the different C/N/P ratio in both wastewaters (C/N/P of 100/15.6/0.6 in PWW and 100/18/5 in domestic wastewater). Likewise, these results were in agreement with those obtained by Cabanelas et al. (2013), who reported a C, N and P content in the harvested biomass of ≈ 44, 7.5 and 0.5 %, respectively, in a photobioreactor inoculated with *Chlorella vulgaris* and supplemented with CO_2 during the treatment of effluent from primary settler. In this context, the results herein obtained confirmed the similar algal-bacterial biomass composition regardless of the microalgae species present in the cultivation broth or operational conditions.

.

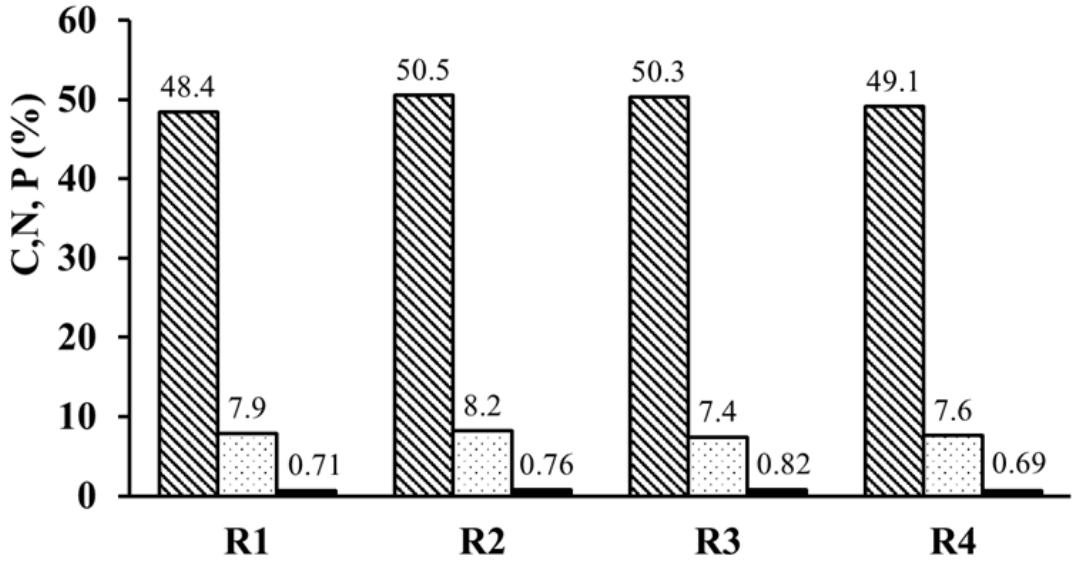


Figure 5. C (▨), N (⬚) and P (■) content in the biomass present in the photobioreactors under steady state.

3.4 Heavy metals removal efficiency

The overall steady state Zn-REs in R1, R2, R3 and R4 accounted for 49±6, 37±6, 26±5 and 49±5 %, respectively, which resulted in average effluent Zn concentrations of 0.9±0.2, 1.1±0.1, 1.3±0.3 and 0.9±0.3 mg/L, respectively, at the end of the operational period (Table 1). These values were similar (Zn-REs of 37%) to those reported by Abe et al. (2008) during PWW treatment in wetlands. The fact that the highest Zn-REs occurred in the photobioreactors with the highest biomass concentrations (R1 and R4) and the lowest Zn-RE in R3 (at the lowest biomass concentration) suggested that Zn removal was mediated by biosorption onto the algal-bacterial biomass present in the photobioreactor (Table 1) (Kaplan et al., 1987; Muñoz et al., 2006). This showed the high tolerance of species such as *Chlorella* sp. to heavy metal contamination (Muñoz et al., 2006). Higher Zn-REs by biosoportion would be expected at higher pHs according to Muñoz et al. (2006), who observed an increase in Zn accumulation into the algal-bacterial biomass from 5.0 to 11.7 mg Zn/g biomass when pHs was raised from 7 to 9, respectively. The determination of copper and arsenic removal efficiencies was not possible based on the low concentrations of these metals in the PWW (below the detection limit of the instrument = 0.6 mg/L).

4. Conclusion

This research revealed the difficulty to maintain monoalgal cultures during PWW treatment in open-photobioreactors operated under similar environmental and operational conditions. The high abundance of *Chlorella* sp. in most photobioreactors confirmed the high tolerance of this microalga to the pollutants. The acclimation of native species to the characteristics of the PWW resulted in highest biomass concentrations. An efficient PWW treatment occurred regardless of the microalgae species inoculated, which confirmed the robustness of algal-bacterial processes devoted to carbon and nutrient removals from livestock wastewaters. Finally, the heavy metals can be removed by biosorption into the algal-bacterial biomass produced during PWW bioremediation.

Acknowledgments

This research was supported by INIA, the European FEDER program (RTA2013-00056-C03-02), the Regional Government of Castilla y León (Project VA024U14 and UIC 71) and MINECO (Red Novedar). The financial support of the EU program ERASMUS MUNDUS EURICA and Universidad Nacional Autónoma de Nicaragua (UNAN-Managua) are also gratefully acknowledged.

References

1. Abe, K., Waki, M., Suzuki, K., Kasuya, M., Suzuki, R., Itahashi, S., Banzai, K., 2012. Estimation of Zn and Cu unit output loads from animal husbandry facilities. Water Sci. Technol. 66, 653–658. doi:10.2166/wst.2012.224

2. Abou-Shanab, R.A.I., Ji, M.-K., Kim, H.-C., Paeng, K.-J., Jeon, B.-H., 2013. Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. J. Environ. Manage. 115, 257–264. doi:10.1016/j.jenvman.2012.11.022

3. Acién, F.G., Gómez-Serrano, C., Morales-Amaral, M.M., Fernández-Sevilla, J.M., Molina-Grima, E., 2016. Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? Appl. Microbiol. Biotechnol. 100, 9013–9022. doi:10.1007/s00253-016-7835-7

4. APHA, 2005. Standards Methods for the Examination of Water and Wastewater, 21 st. ed. American Public Health Association, American Water Works Association, Water Enviroment Federation, Washington,D.C.

5. Bastos, R.G., Bonini, M. a., Zepka, L.Q., Jacob-Lopes, E., Queiroz, M.I., 2014. Treatment of rice parboiling wastewater by cyanobacterium Aphanothece microscopica Nägeli with potential for biomass products. Desalin. Water Treat. 3994, 1–7. doi:10.1080/19443994.2014.937758

6. Cabanelas, I.T.D., Ruiz, J., Arbib, Z., Chinalia, F.A., Garrido-Pérez, C., Rogalla, F.,

Nascimento, I.A., Perales, J.A., 2013. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresour. Technol. 131, 429–436. doi:10.1016/j.biortech.2012.12.152

7. Chandrasekhar, K., Lee, Y.-J., Lee, D.-W., 2015. Biohydrogen Production: Strategies to Improve Process Efficiency through Microbial Routes. Int. J. Mol. Sci. Int. J. Mol. Sci 16, 8266–8293. doi:10.3390/ijms16048266

8. Cheah, W.Y., Ling, T.C., Show, P.L., Juan, J.C., Chang, J.S., Lee, D.J., 2016. Cultivation in wastewaters for energy: A microalgae platform. Appl. Energy 179, 609–625. doi:10.1016/j.apenergy.2016.07.015

9. De Godos, I., Blanco, S., García-Encina, P.A., Becares, E., Muñoz, R., 2009. Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates. Bioresour. Technol. 100, 4332–4339. doi:10.1016/j.biortech.2009.04.016

10. De la Torre, A.I., Jiménez, J.A., Carballo, M., Fernandez, C., Roset, J., Muoz, M.J., 2000. Ecotoxicological evaluation of pig slurry. Chemosphere 41, 1629–1635. doi:10.1016/S0045-6535(00)00038-2

11. Ehimen, E.A., Sun, Z.F., Carrington, C.G., Birch, E.J., Eaton-Rye, J.J., 2011. Anaerobic digestion of microalgae residues resulting from the biodiesel production process. Appl. Energy 88, 3454–3463. doi:10.1016/j.apenergy.2010.10.020

12. EU, 2016. https://ec.europa.eu/info/legal-notice_en [WWW Document]. URL http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=apro_mt_lspig&lang=en (accessed 11.7.16).

13. EU, 2015. http://ec.europa.eu [WWW Document]. URL http://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_animals (accessed 1.10.17).

14. Franchino, M., Tigini, V., Varese, G.C., Mussat Sartor, R., Bona, F., 2016. Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. Sci. Total Environ. 569, 40–45. doi:10.1016/j.scitotenv.2016.06.100

15. García, D., Alcántara, C., Blanco, S., Pérez, R., Bolado, S., Muñoz, R., 2017. Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading. Chem. Eng. J. 313, 424–434. doi:10.1016/j.cej.2016.12.054

16. González-Fernández, C., Sialve, B., Bernet, N., Steyer, J.., 2012a. Impact of microalgae characteristic on their conversion to biofuel. Part I: Focus on cultivation and biofuel production. Biofuels, Bioprod. Biorefining 105–113. doi:10.1002/bbb

17. González-Fernández, C., Sialve, B., Bernet, N., Steyer, J.., 2012b. Impact of microalgae characteristics on their conversion to biofuel. Part II: Focus on biomethane production. Biofuels, Bioprod. Biorefining 205–2018. doi:10.1002/bbb

18. Guieysse, B., Béchet, Q., Shilton, A., 2013. Variability and uncertainty in water demand and water footprint assessments of fresh algae cultivation based on case studies from five climatic regions. Bioresour. Technol. 128, 317–323. doi:10.1016/j.biortech.2012.10.096

19. Kaplan, D., Cristiaen, D., Shoshana, A., 1987. Chelating Properties of Extracellular Polysaccharides from Chlorella spp. Appl. Environ. Microbiol. 53, 2953–2956.

20. Kim, H.-C., Choi, W.J., Chae, A.N., Park, J., Kim, H.J., Song, K.G., 2016. Treating

high-strength saline piggery wastewater using the heterotrophic cultivation of Acutodesmus obliquus. Biochem. Eng. J. 110, 51–58. doi:10.1016/j.bej.2016.02.011

21. Kuo, C.M., Chen, T.Y., Lin, T.H., Kao, C.Y., Lai, J.T., Chang, J.S., Lin, C.S., 2015. Cultivation of Chlorella sp. GD using piggery wastewater for biomass and lipid production. Bioresour. Technol. 194, 326–333. doi:10.1016/j.biortech.2015.07.026

22. MAGRAMA, 2015. Censo y producción: Producción de porcino ibérico [WWW Document]. URL http://www.magrama.gob.es/es/ganaderia/temas/produccion-y-mercados-ganaderos/sectores-ganaderos/porcino/ (accessed 10.18.16).

23. Muñoz, R., Alvarez, M.T., Muñoz, A., Terrazas, E., Guieysse, B., Mattiasson, B., 2006. Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. Chemosphere 63, 903–11. doi:10.1016/j.chemosphere.2005.09.062

24. Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: A review. Water Res. 40, 2799–2815. doi:10.1016/j.watres.2006.06.011

25. Muñoz, R., Köllner, C., Guieysse, B., Mattiasson, B., 2003. Salicylate biodegradation by various algal-bacterial consortia under photosynthetic oxygenation. Biotechnol. Lett. 25, 1905–1911. doi:10.1023/B:BILE.0000003980.96235.fd

26. Naik, S.N., Goud, V. V., Rout, P.K., Dalai, A.K., 2010. Production of first and second generation biofuels: A comprehensive review. Renew. Sustain. Energy Rev. 14, 578–597. doi:10.1016/j.rser.2009.10.003

27. Olguín, E.J., Mendoza, A., González-Portela, R.E., Novelo, E., 2013. Population dynamics in mixed cultures of Neochloris oleoabundans and native microalgae from water of a polluted river and isolation of a diatom consortium for the production of lipid rich biomass. N. Biotechnol. 30, 705–715. doi:10.1016/j.nbt.2013.03.003

28. Palmer, C.M., 1969. A composite rating of algae tolerating organic pollution. J. Phycol. 5, 78–82. doi:10.1111/j.1529-8817.1969.tb02581.x

29. Park, J.B.K., Craggs, R.J., Shilton, A.N., 2011. Recycling algae to improve species control and harvest efficiency from a high rate algal pond. Water Res. 45, 6637–6649. doi:10.1016/j.watres.2011.09.042

30. 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. doi:10.1016/j.biortech.2013.04.008

31. Posadas, E., Marín, D., Blanco, S., Lebrero, R., Muñoz, R., 2017. Simultaneous biogas upgrading and centrate treatment in an outdoors pilot scale high rate algal pond. Bioresour. Technol. doi:10.1016/j.biortech.2017.01.071

32. Posadas, E., Morales, M.D.M., Gomez, C., Acién, F.G., Muñoz, R., 2015. Influence of pH and CO2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chem. Eng. J. 265, 239–248. doi:10.1016/j.cej.2014.12.059

33. 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. doi:10.1016/j.algal.2015.09.002

34. Queiroz, M.I., Lopes, E.J., Zepka, L.Q.,

Bastos, R.G., Goldbeck, R., 2007. The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor. Bioresour. Technol. 98, 2163–2169. doi:10.1016/j.biortech.2006.08.034

35. Richmond, A., 2004. Handbook of microalgal culture: biotechnology and applied phycology/edited by Amos Richmond. doi:10.1002/9780470995280

36. Serejo, M.L., Posadas, E., Boncz, M.A., Blanco, S., García-Encina, P., Muñoz, R., 2015. Influence of Biogas Flow Rate on Biomass Composition During the Optimization of Biogas Upgrading in Microalgal-Bacterial Processes. Environ. Sci. Technol. 3228–3236. doi:10.1021/es5056116

37. Sournia, A., 1978. Phytoplankton manual. UNESCO, Paris.

38. Talbot, P., Thébault, J.., Dauta, A., De la Noüe, J., 1991. A comparative study and mathematical modeling of temperature, light and growth of three microalgae potentially useful for wastewater treatment. Water Res. 25, 465–472. doi:10.1016/0043-1354(91)90083-3

39. Vimalarasan, A., Pratheeba, N., Ashokkumar, B., Sivakumar, N., Varalakshmi, P., 2011. Production of biodiesel from cyanobacteria (Oscillatoria annae) by alkali and enzyme mediated transesterification. J. Sci. Ind. Res. (India). 70, 959–967.

40. Yuan, Z., Wang, Z., Takala, J., Hiltunen, E., Qin, L., Xu, Z., Qin, X., Zhu, L., 2013. Scale-up potential of cultivating chlorella zofingiensis in piggery wastewater for biodiesel production. Bioresour. Technol. 137, 318–325. doi:10.1016/j.biortech.2013.03.144

SUPPLEMENTARY MATERIALS

Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors

Dimas García[1,2], Esther Posadas[1], Saúl Blanco[3], Gabriel Acién[4], Pedro A. García Encina[1], Silvia Bolado[1], Raúl Muñoz[1]*

[1]Department of Chemical Engineering and Environmental Technology, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

[2]Centro para la Investigación de los Recursos Acuáticos de Nicaragua, CIRA/UNAN-Managua, Apdo. Postal 4598

[3]The Institute of the Environment, La Serna 58 - 24007 León, Spain

[4]Department of Chemical Engineering, University of Almeria, Cañada San Urbano, s/n, 04120 Almeria, Spain

*corresponding author: mutora@iq.uva.es

CONTENT

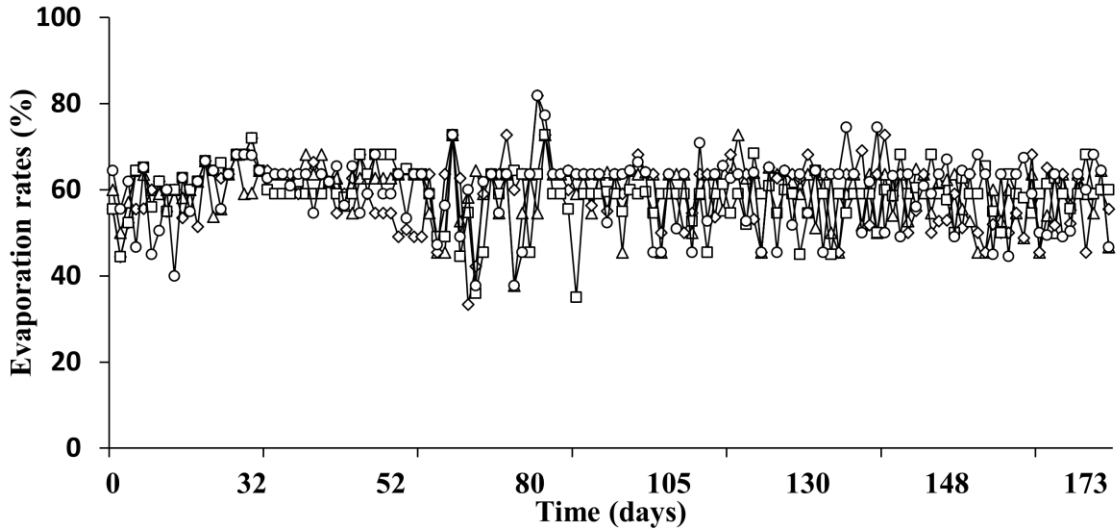


Figure S1. Time course of the evaporation rates in R1 (Δ), R2 (◊), R3 (□) and R4 (○) for the entire experiment.

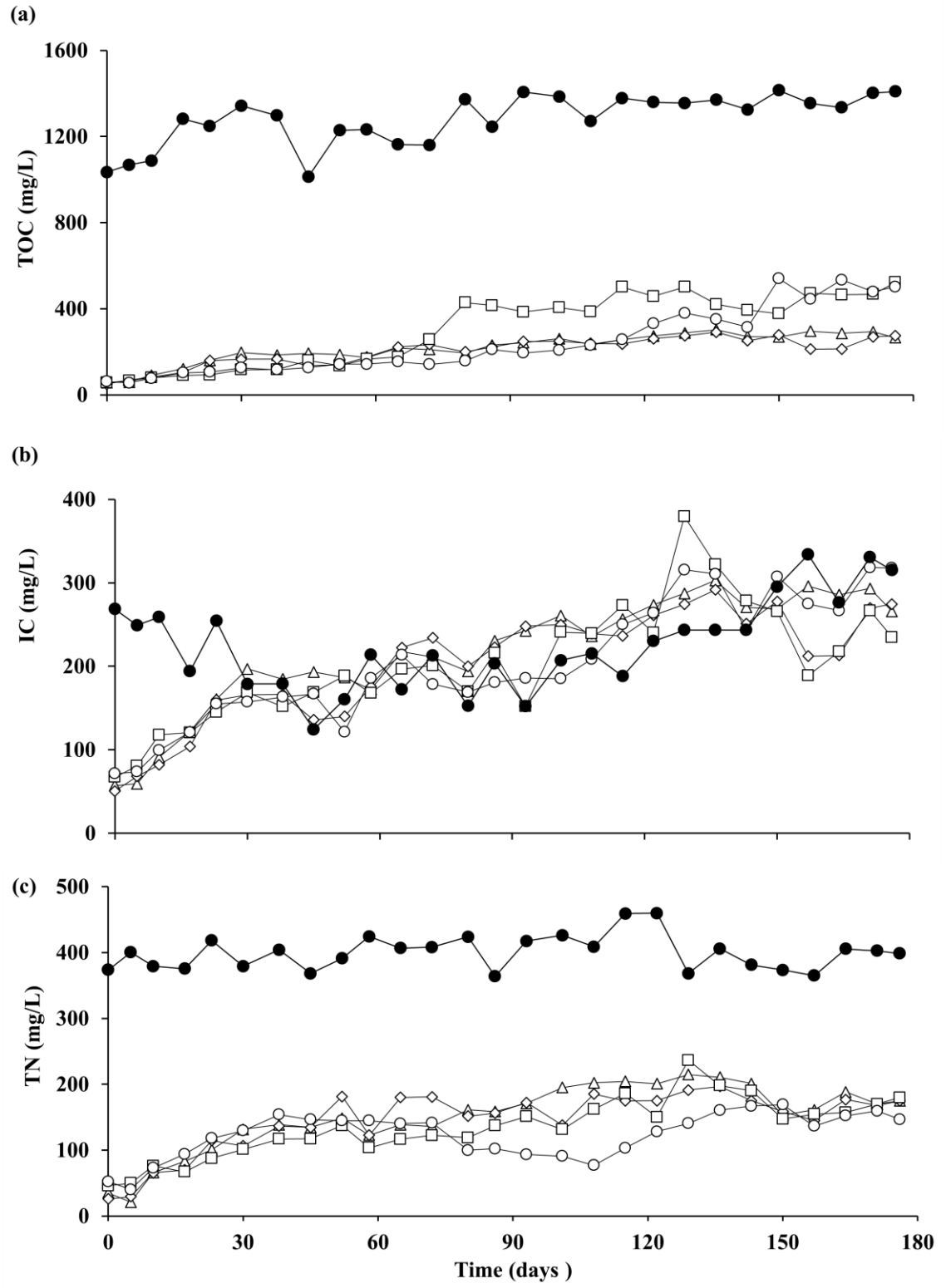


Figure S2. Time course of the concentration of TOC (**a**), IC (**b**) and TN (**c**) in the PWW (●), R1 (Δ), R2 (◊), R3 (□) and R4 (○) for the entire experiment.

Table S1. Operational parameters and photobioreactor dimensions.

HRT (d)	**Q*feed* (L/d)**	**Q*eff* (L/d)**	**% Evaporation**	**V PBR (L)**	**Height PBR (cm)**	**Area PBR (m^2)**
27	0.112	0.044	60	3.0	15.8	0.019

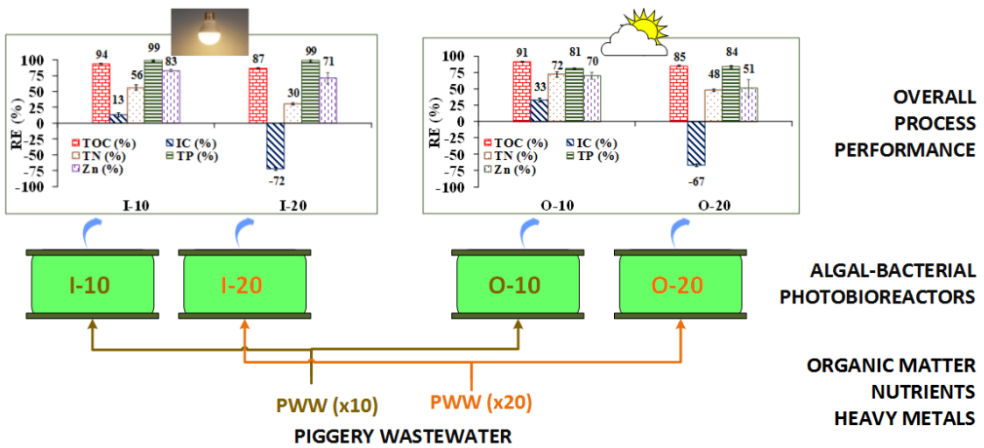
Table S2. Mass balance calculations of PWW treatment under steady state conditions.

Parameter	PWW	R1	R2	R3	R4
TOC (mg/L)	1375	459	452	482	490
TOC (mg/d) in		153	153	153	153
TOC (mg/d) out		20.4	20.1	21.4	21.8
% TOC RE		87	87	86	86
IC (mg/L)	314	285	242	227	294
IC (mg/d) in		35	35	35	35
IC (mg/d) out		12.7	10.8	10.1	13.1
% IC RE		64	69	71	63
TN (mg/L)	393	174	166	165	149
TN (mg/d) in		44	44	44	44
TN (mg/d) out		7.7	7.4	7.3	6.6
% TN RE		82	83	83	85
Nitrite (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Nitrate (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
TP (mg/L)	9.4	2.40	2.10	1.90	1.80
TP (mg/d) in		1.0	1.0	1.0	1.0
TP (mg/d) out		0.1	0.1	0.1	0.1
% TP RE		90	91	92	92
Zinc (mg/L)	0.7	0.9	1.1	1.3	0.9
Zn (mg/d) in		0.08	0.08	0.08	0.08
Zn (mg/d) out		0.04	0.05	0.06	0.04
% Zn RE		49	37	26	49
Copper (mg/L)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
Arsenic (mg/L)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
TSS (mg/L)	1340	2610	2569	2445	3265
Productivity ($g/m^2/d$)		6.1	6.0	5.7	7.6
C %		48.4	50.5	50.3	49.1
C (mg/d) biomass		56.1	57.7	54.7	71.2
% Carbon recovered		36	37	35	47
N %		7.9	8.2	7.4	7.6
N (mg/d) biomass		9.2	9.4	8.0	11.0
% Nitrogen recovered		26	26	22	30
P%		0.7	0.8	0.8	0.7
P (mg/d) biomass		0.8	0.9	0.9	1.0
% Phosphorus recovered		88	91	93	100

Chapter 3

Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions

García, D., Posadas, E., Grajera, C., Blanco, S., Martínez-Páramo, S., Acién, G., García-Encina, P., Bolado, S., Muñoz, R. 2017. Bioresource Technology. 245 (Pt A), 483 - 490. doi.10.1016/j.biortech.2017.08.135. Accepted 22 August 2017



Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions.

Dimas García[1,2], Esther Posadas[1], Carlos Grajeda[1], Saúl Blanco[3], Sonia Martínez-Páramo[1], Gabriel Acién[4], Pedro García-Encina[1], Silvia Bolado[1], Raúl Muñoz[1]*

[1]Department of Chemical Engineering and Environmental Technology, School of Industrial Engineerings, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

[2]Centro para la Investigación de los Recursos Acuáticos de Nicaragua, CIRA/UNAN-Managua, Apdo. Postal 4598, Nicaragua

[3]The Institute of the Environment, La Serna 58 - 24007 León, Spain

[4]Department of Chemical Engineering, University of Almeria, Cañada San Urbano, s/n, 04120 Almeria, Spain

*corresponding author: mutora@iq.uva.es

Abstract

This work evaluated the performance of four open algal-bacterial photobioreactors operated at ≈ 26 days of hydraulic retention time during the treatment of 10 (×10) and 20 (×20) times diluted piggery wastewater (PWW) under indoor (I) and outdoor (O) conditions for four months. The removal efficiencies (REs) of organic matter, nutrients and zinc from PWW, along with the dynamics of biomass concentration and structure of algal-bacterial population were assessed. The highest TOC-RE, TP-RE and Zn-RE (94±1%, 100% and 83±2%, respectively) were achieved indoors in ×10 PWW, while the highest TN-RE (72±8%) was recorded outdoors in ×10 PWW. *Chlorella vulgaris* was the dominant species regardless of the ambient conditions and PWW dilution. Finally, DGGE-sequencing of the bacterial community revealed the occurrence of four phyla, *Proteobacteria* being the dominant phylum with 15 out of the 23 most intense bands.

Keywords

Algal-bacterial processes;
Heavy metal biosorption;
Microalgae-bacteria dynamics;
Nutrient removal;
Piggery wastewater biodegradation.

1. Introduction

Europe, with an annual production of 23.5 million tn of pork meat, was the second largest pig producer in the world in 2015 (Statista, 2016). Europe´s pig production accounted for 149 million heads, which represented approx. 44.3 % of the total European livestock in 2015 (EU, 2015; MAGRAMA, 2015). However, this relevant economic sector annually generates 217-434 million m^3 of piggery wastewater (PWW) (4-8 L/d·pig) containing high concentrations of organic matter, nutrients, solids and heavy metals (De Godos et al., 2009; Franchino et al., 2016). The treatment of such high strength wastewaters represents both a technical challenge and a severe economic burden for the livestock sector. In this context, next generation PWW treatment technologies should allow complying with European wastewater regulations (1999/31/EC) (Council Directive, 1999) while producing added-value bioproducts out of the organic matter and nutrients present in PWW (2008/98/EC) (European Commisssion, 2008).

Algal-bacterial symbiosis has emerged as a promising platform for resource recovery and recycling from PWW in rural areas (where space is often not limiting). Algal-bacterial symbiosis has been successfully applied in photobioreactors for the treatment of domestic wastewater (García et al., 2017a; Oswald et al., 1957), digestates (Anbalagan et al., 2016; Wang et al., 2013), livestock effluents (Tigini et al., 2016), parboiled rice wastewater (Bastos et al., 2009), olive oil mill wastewater and wastewater from the pulp and paper industry (Muñoz and Guieysse, 2006). The use of microalgae during PWW treatment can support a cost-effective removal of organic matter, nutrients, heavy metals, pathogens and emerging pollutants as a result of their dual autotrophic and heterotrophic metabolisms, photosynthetic O_2 release and ability to increase the pH of the cultivation broth (García et al., 2017a; Muñoz and Guieysse, 2006). The ability of microalgae to grow on both wastewater alkalinity and the carbon dioxide (CO_2) released during organic matter oxidation entails 2-3 folds larger productivities (compared to activated sludge systems) of a biomass that can be used as a feedstock for the production of biofertilizers or bioenergy. In addition, the lower energy demand of microalgae-based wastewater treatment, along with the CO_2 fixation ability of microalgae, significantly increase the environmental sustainability of this technology (Cheah

et al., 2016; Dassey and Theegala, 2013). Despite the merits of algal-bacterial processes for PWW treatment and the intensive research conducted in this field in the past 10 years, very few studies have been carried out outdoors under the periodically fluctuating and high solar irradiations and temperatures (De Godos et al., 2009; García et al., 2017b; Posadas et al., 2017). In this context, the absence of comparative studies systematically assessing the representativeness of the results obtained indoors (under artificial irradiation and temperature controlled environments) compared to those supported by outdoors photobioreactors severely limits the use of most data available in literature for the design and operation of full-scale microalgae-based systems.

This work aimed at systematically evaluating the potential of open algal-bacterial photobioreactors for the treatment of PWW under indoor and outdoor conditions. The removal of carbon, nitrogen, phosphorus and heavy metals was assessed at two PWW dilutions under solar and artificial illumination. Finally, the influence of both PWW dilution and environmental conditions on the structure of the microalgae and bacteria communities was investigated.

2. Materials and methods

2.1 *Algal-bacterial inoculum and piggery wastewater*

An acclimated *Chlorella vulgaris* culture, obtained from an indoor open algal-bacterial photobioreactor treating 15% diluted PWW at the Department of Chemical Engineering and Environmental Technology at Valladolid University (Spain), was used as inoculum. Fresh PWW was collected from a nearby farm at Cantalejo (Spain) and stored at 4 °C. The PWW was centrifuged for 10 minutes at 10000 rpm before dilution to reduce the concentration of suspended solids. The average composition of the 10 and 20 folds diluted PWW is shown in Table 1.

2.2 Experimental system

The indoors experimental set-up consisted of two 3 L open photobioreactors (15.8 cm depth, 15.5 cm internal diameter) illuminated at 1417±82 μmol/m^2·s for 12 hours a day (08h00 to 20h00) by LED lamps arranged in a horizontal configuration 60 cm above the photobioreactor surface under indoor conditions (Fig. 1, Table 1). Likewise, two similar open photobioreactors were located outdoors at the Department of Chemical Engineering and Environmental

Technology at Valladolid University (Spain). The average photosynthetic active radiation (PAR) in these systems at 11h00 was 1394±171 μmol/m^2·s (Fig. 1, Table 1). This value was comparable to the daily average PARs provided by the official AEMET meteorological station located at the University of Valladolid during the experimental period (1210±126 μmol/m^2·s). The temperature of the indoor and outdoor photobioreactors was partially controlled using a water bath to prevent the high temperatures induced by both LEDs and solar irradiation. The algal-bacterial cultivation broth in the photobioreactors was gently mixed via water immersion pumps. The indoors and outdoors photobioreactors were fed with both 10 and 20 times diluted PWW using an auto control 205U7CA multi-channel cassette pump (Watson-Marlow, UK). PWW dilutions were selected based on previous investigations carried out with this kind of wastewater and aiming to avoid microbial inhibition as a consequence of PWW toxicity (De Godos et al., 2009; García et al., 2017b; González et al., 2008). Pure CO_2 was added to the cultivation broth of the phobioreactors to automatically maintain the pH at 8.0 using a Crison multimeter M44 control unit (Crison Instruments, Spain).

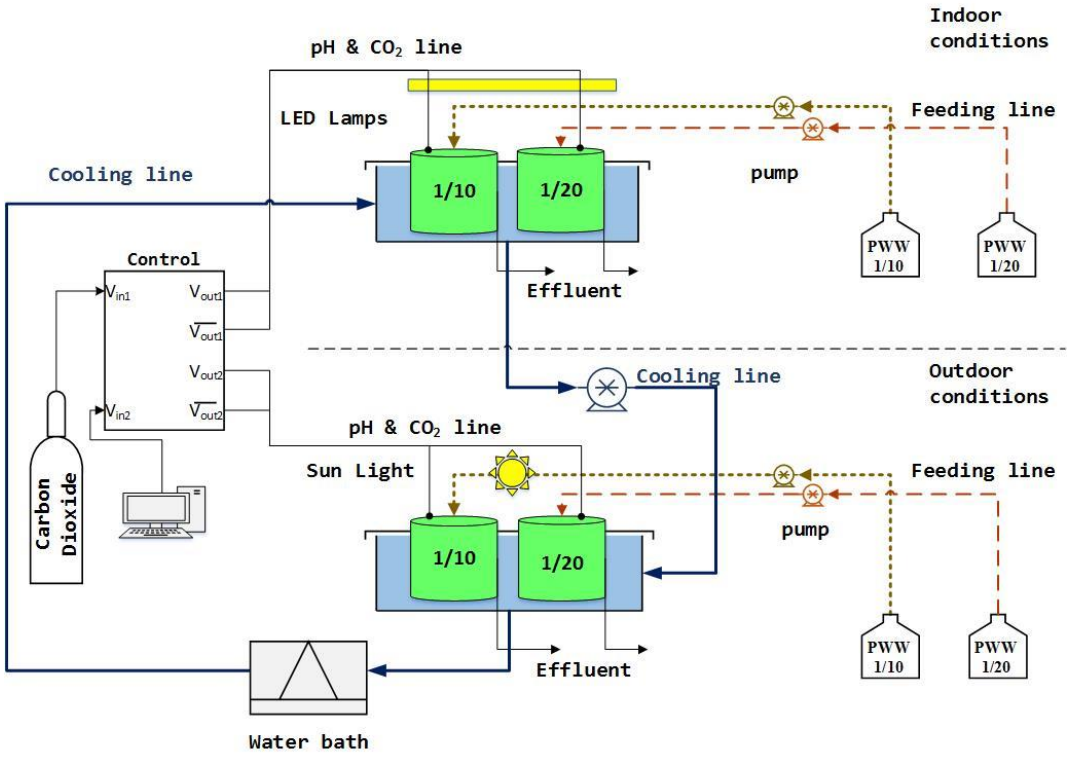


Figure 1. Schematic diagram of the algal-bacterial photobioreactor set-up equipped with carbon dioxide supplementation for pH control under indoor and outdoor conditions.

2.3 Experimental design and sampling procedure

The indoors photobioreactors fed with 10 and 20 times diluted PWW (namely I-10 and I-20, respectively) and the outdoors photobioreactors fed with 10 and 20 times diluted PWW (namely O-10 and O-20, respectively) were inoculated with a fresh *Chlorella vulgaris* culture at an initial TSS concentration of ≈ 680 mg/L (corresponding to an initial microalgae cell concentration of ≈ $1.06 \cdot 10^9$ cells/L, respectively). The photobioreactors, which were initially filled with tap water, were operated at an average hydraulic retention time (HRT) of ≈ 26 days for 120 days (from May-2016 to Sept-2016). A higher HRT than in conventional HRAPs (3-10 days) was chosen in this research to guarantee an effective carbon and nutrients removal, and to prevent toxicity effects on microbial population due the high loads of organic matter and nutrients of the PWW treated in this study (Aguirre et al., 2011; De Godos et al., 2009). The effluent from the photobioreactors overflowed separately as a function of the evaporation rates. Liquid samples from the influent PWWs and effluents of the photobioreactors were taken weekly to determine the concentration of total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), zinc (Zn) and total suspended solid (TSS). Likewise, the structure of the microalgae population in the photobioreactors was periodically assessed from biomass samples preserved with lugol acid at 5% and formaldehyde at 10%, and stored at 4 ºC prior to analysis. A cultivation broth sample from the photobioreactors was also collected under steady state (day 120) and immediately stored at -20 ºC to evaluate the richness and composition of the bacterial communities (Alcántara et al., 2015). Dissolved oxygen (DO) concentration and temperature in the photobioreactors were measured twice per day (11h00 and 17h00), while the influents and effluents flowrates were daily recorded to monitor water evaporation losses (Table 1). Finally, the C, N and P content of the algal-bacterial biomass present in the photobioreactors was measured under steady state.

The removal efficiencies of C, N, P and Zn were calculated according to Eq. (1):

$$RE(\%) = \frac{(C_{feed} \times Q_{feed}) - (C_{eff} \times Q_{eff})}{C_{feed} \times Q_{feed}} \times 100 \quad (1)$$

where C_{feed} and C_{eff} represent the dissolved concentrations of TOC, IC, TN, TP and Zn in the influent PWWs and photobioreactors effluents, respectively, while Q_{feed} and Q_{eff} represent the PWWs and effluents flow rate, respectively. The mass flow rate of C-CO_2 injected to control the pH was negligible compared to the input mass flow rate of C in the influent PWW (data not shown). The process was considered under steady state when the TSS concentrations in the photobioreactors remained stable for at least four consecutive samplings (~ 1 month). The results obtained were here provided as the average ± standard deviation from duplicate measurements along the one-month steady state period (days 91-120).

2.4 Analytical procedures

pH was in-situ measured using a Crison M44 multimeter and a Crison PH 28 meter. An OXI 330*i* oximeter was used to measure the DO and temperature (WTW, Germany). A LI-250A light meter (LI-COR Biosciences, Germany) was used to measure the light intensity as PAR. TOC, IC and TN concentrations were determined using a TOC-V CSH analyzer equipped with a TNM-1 module (Shimadzu, Japan). Nitrate and nitrite were analyzed by high performance liquid chromatography-ion conductivity (HPLC-IC) (Posadas et al., 2013). N-NH_4^+ was not analyzed based on the fact that no inhibition was expected at a pH of 8.0, where the ammonium share is greater than > 90% of the total nitrogen (Metcalf and Eddy, 2003). TP and TSS concentrations were determined according to Standard Methods (APHA, 2005). The analysis of the C, N and P biomass content in pre-dried and grinded algal-bacterial biomass was carried out using a LECO CHNS-932 elemental analyzer. Zinc was determined using a 725-ICP Optical Emission Spectrophotometer (Agilent, USA) at 213.62 nm. The concentrations of arsenic and copper were not determined based on the results obtained by (García et al., 2017b), who observed that the concentration of these heavy metals in this PWW always remained below the detection limit (< 0.6 mg/L).

The identification and quantification of microalgae were conducted by microscopic examination (OLYMPUS IX70, USA) according to Sournia (1978). Molecular analysis of the bacterial populations was carried out according with Frutos et al. (2015). The genomic **desoxyribonucleic acid** (DNA) was extracted using the protocol described in the Fast® DNA Spin Kit for Soil (MP Biomedicals, LLC) handbook.

The genes in the V6-V8 regions of the bacterial 16S ribosomal ribonucleic acid (rRNA) were amplified by Polymerase Chain Reaction (PCR) analysis using the universal bacterial primers 968-F-GC and 1401-R (Sigma-Aldrich, St. Louis, MO, USA) (Nübel et al., 1996). The denaturing gradient gel electrophoresis (DGGE) analysis of the amplicons was performed with a D-Code universal mutation system (Bio Rad Laboratories) using 8 % (w/v) polyacrylamide gels with a urea/formamide denaturing gradient of 45 to 65 %. DGGE running conditions were applied according to Roest et al. (2005). Sequences were deposited in GenBank Data Library under accession numbers MF380643 al MF380665. The Shannon-Wiener diversity index (H) was determined using the peak heights in the densitometric curves. This index, which reflects both the sample richness and evenness and ranges from 1.5 to 3.5 (low and high species evenness and richness, respectively), can be calculated according to Eq. (2) (MacDonald, 2003):

$$H = -\Sigma\ [P_i ln(P_i)] \quad (2)$$

Where H is diversity index and P_i is the importance probability of the bands in a lane ($P_i = n_i/n$, where n_i is the height of an individual peak and n is the sum of all peak heights in the densitometric curves). Similarity indices of the compared profiles were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient (Häne et al., 1993). The taxonomic position of the sequenced DGGE bands was obtained using the RDP classifier tool (50 % confidence level) (Wang et al., 2007). The closest cultured and uncultured relatives to each band were obtained using the BLAST search tool at the NCBI database (National Centre for Biotechnology Information) (McGinnis and Madden, 2004).

Table 1. Operational conditions and physical/chemical characterization of the piggery wastewater (PWW) and cultivation broth in the photobioreactors.

Parameters		PWW (×10)	PWW (×20)	I-10	I-20	O-10	O-20
Operation period (days)		*	*	120	120	120	120
HRT (days)		*	*	≈26	≈26	≈26	≈26
pH (units)		*	*	8.0	8.0	8.0	8.0
PAR (µmol/m^2.s)		*	*	1417±82	1417±82	1394±171	1394±171
Temperature (◦C)	11h00	*	*	21±4	21±4	26±5	26±5
	17h00	*	*	24±4	24±4	35±5	35±5
Dissolved Oxygen (mg/L)	11h00	*	*	3.5±2.2	6.1±4.4	1.9±0.8	3.9+1.7
	17h00	*	*	2.3±1.7	5.8±4.8	1.3+0.3	2.7±1.2
Evaporation rates (%)		*	*	27	27	44	44
TOC (mg/L)		963±71	497±33	80±5	91±5	150±11	133±8
IC (mg/L)		160±15	82±2	188±3	191±5	191±9	241±6
TN (mg/L)		341±27	170±3	201±6	162±5	168±6	158±7
Nitrate (mg/L)		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Nitrite (mg/L)		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
TP (mg/L)		4.9±0.2	2.5±0.1	0.07±0.01	0.04±0.01	1.67±0.08	0.70±0.07
Zinc (mg/L)		0.66±0.04	0.37±0.02	0.16±0.02	0.15±0.05	0.35±0.03	0.32±0.08
TSS (mg/L)		291±3	156±3	1284±71	720±16	1328±28	655±13
*Not applicable							

3. Results and Discussion

3.1 Biodegradation of carbon, nitrogen and phosphorous

The range of temperatures, DO and PAR along with pH control resulted in a successful PWW treatment regardless of the environmental and operational conditions imposed (Table 1, Fig. 2). The higher evaporation rates under outdoor conditions (44% of the influent PWW flowrate compared to 27% under indoor conditions) resulted in a significant deterioration of the quality of the treated effluent (Table 1).

The TOC-REs accounted for 94±1, 87±2, 91±1 and 85±1% in I-10, I-20, O-10 and O-20, respectively, which resulted in average TOC concentrations in the effluent at the end of the operational period of 80±5, 91±5, 150±11 and 133±8 mg/L, respectively (Table 1, Fig. 2). These high REs were supported by the DOs > 1 mg O_2/L in the cultivation broth of the four photobioreactors mediated by an intense photosynthetic activity (Table 1). The slightly higher TOC-REs during the treatment of 10 times diluted PWW regardless of the environmental conditions can be explained by the differences in microbial population structure and biomass concentration encountered in the photobioreactors under steady state. Thus, a higher share, diversity and concentration of bacteria compared to microalgae was present in the photobioreactors supplied with 10 times diluted PWW as revealed by the TSS measurements, DGGE analyses and microalgae population characterization. (Table 1). On the other hand, the higher DO concentrations recorded in the indoor photobioreactors regardless of the organic loading rate applied were likely caused by the lower temperatures (mediating a higher O_2 solubility and a lower bacterial activity) and by the constant PAR (resulting in a higher microalgal activity) (Posadas et al., 2015). The results here obtained were in agreement with the organic matter removal efficiencies reported by De Godos et al. (2009) under outdoor conditions in a 464 L HRAP operated at 10 days of HRT (COD-REs of 76±11% during the treatment of 10 and 20 folds diluted PWW). Likewise, Aguirre et al. (2011) recorded COD-REs ≥ 90 % during the treatment of raw PWW in a 400 L HRAPs under outdoor environmental conditions at a HRT of 40 days. Finally, IC-REs of 13±3, -72±2, 33±2 and -67±13% were recorded under steady state in I-10, I-20, O-10 and O-20, respectively, which resulted in average IC concentrations in the effluent of

188±3, 191±5, 191±9 and 241±6 mg/L, respectively (Table 1, Fig. 2). The negative IC-REs recorded in I-20 and O-20 resulted from the accumulation of inorganic carbon mediated by the high TOC oxidation activity in the systems, which was in agreement with the results obtained by (Posadas et al., 2013). The higher IC-REs during the treatment of 10 times diluted PWW were likely supported by the higher biomass concentrations (≈1300 mg TSS/L in I-10 and O-10 compared to ≈700 mg TSS/L in I-20 and O-20). In this context, process operation at high biomass concentrations in photobioreactors under high PARs can prevent microalgae photoinhibition and thus induce high photosynthetic activities. Carbon removal by stripping (prior mineralization of the organic carbon to CO_2) was the main mechanism accounting for carbon removal in I-10 and O-10, since only 49 and 37 % of the total carbon removed was recovered in the form of harvested biomass, respectively.

The TN-REs accounted for 56±4, 30±14, 72±8 and 48±9% in I-10, I-20, O-10 and O-20, respectively, which resulted in average TN concentrations in the effluent under steady state conditions of 201±6, 162±5, 168±6 and 157±7 mg/L, respectively (Table 1, Fig. 2). The higher temperatures prevailing outdoors likely lowered NH_3 solubility and therefore increased N removal by stripping (Fig. 2) (Metcalf and Eddy, 2003). In addition, the systems supporting higher biomass concentrations (I-10 and O-10) mediated higher N removals compared to process operation with 20 folds diluted PWW (Fig. 2). The TN-REs here achieved were similar to those obtained by Aguirre et al. (2011) who reported TN-REs ranging from 65 to 85% during PWW treatment in 400 L HRAPs operated at HRTs of 40-80 days, but lower than those reported by García et al. (2017b) (82 - 85%) during the treatment of 15% diluted PWW in open photobioreactors at a HRT of ≈ 27 days operated indoors. The nitrogen mass balances conducted revealed that stripping was the main N removal mechanism in I-10, O-10 and O-20, with nitrogen assimilation into biomass accounting for only 44, 25 and 37% of the total nitrogen removed, respectively. However, nitrogen assimilation was the main N removal mechanism in I-20 (85% of TN removed) likely due to the lower temperatures and TN concentrations prevailing in the cultivation both.

The TP-REs accounted for 99±3, 99±5, 81±8 and 84±13% in I-10, I-20, O-10 and O-20, respectively, which resulted in

average TP concentrations in the effluent of 0.07±0.07, 0.04±0.08, 1.67±0.08 and 0.70±0.07 mg/L, respectively, under steady state (Table 1, Fig. 2). The fact that higher TP-REs were recorded indoors regardless of the PWW dilution and biomass concentration at a constant pH requires further investigation. The TP-REs herein obtained were similar to those reported by García et al. (2017b) during the treatment of 15% diluted PWW in indoors open photobioreactors (90 to 92%). Phosphorous assimilation into algal-bacterial biomass was likely the main removal mechanism based on the moderate pH values prevailing in the photobioreactors during the entire experiment (pH=8.0), which did not support a significant phosphate precipitation (García et al., 2017a). Thus, a phosphorus mass balance revealed that 100, 99, 100 and 100% of the total removed phosphorus was recovered in the harvested biomass in I-10, I-20, O-10 and O-20 respectively.

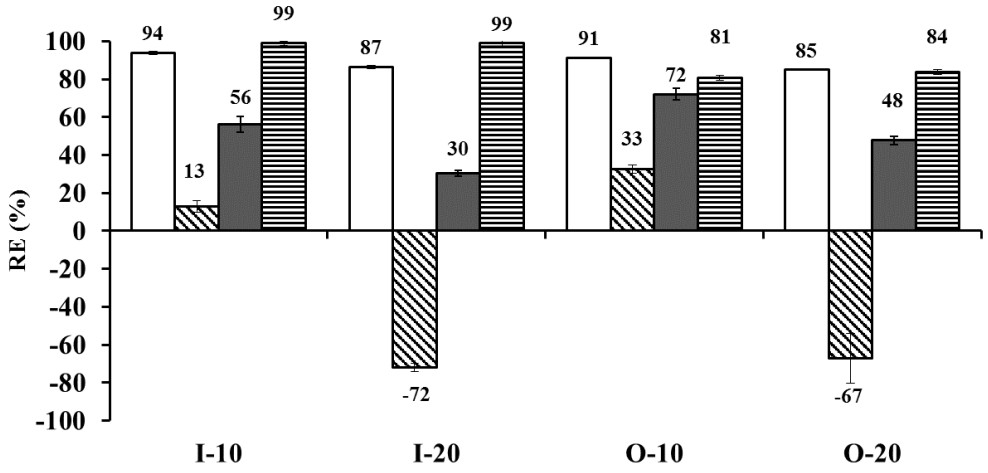


Figure 2. Average removal efficiencies of TOC (▭), IC (▨), TN (▬) and TP (▤) under steady state. Bold numbers indicate the steady state removal efficiencies, while vertical bars represent the standard deviation from replicate measurements during steady state operation.

3.2 Heavy metal removal

The overall steady state Zn-REs in I-10, I-20, O-10 and O-20 accounted for 83±2, 71±9, 70±5 and 51±13%, respectively, which resulted in average Zn concentrations at the end of the operational period of 0.16±0.02, 0.15±0.05, 0.35±0.03 and 0.32±0.08 mg/L, respectively (Table 1). In this context, the higher abundance of microalgae and cyanobacteria induced by laboratory conditions likely supported the higher Zn-REs recorded indoors, while the higher biomass

concentrations in the HRAPs treating 10 folds diluted PWW explained the superior Zn-REs in I-10 and O-10 compared to I-20 and O-20, respectively. The latter suggests that biosorption was the main mechanism governing Zn removal (Javanbakht et al., 2014; Kaplan et al., 1987). The Zn-REs herein obtained were higher than those reported by García et al. (2017b) during PWW treatment in 3 L indoors HRAPs operated at a HRT of ≈27 days (26 to 49%).

3.3 Concentration, productivity and elemental composition of the algal-bacterial biomass

The algal-bacterial biomass concentration in I-10, I-20 and O-20 initially decreased from 680 mg TSS/L to 127, 177 and 170 mg TSS/L, respectively, during the first 28 days of operation, while biomass concentration slightly increased from 680 to 800 mg TSS/L in O-10 during process start-up (Fig. 3). The previous acclimation of microalgae to the pollutants loading rate and environmental conditions imposed to I-20 explain this increase in biomass concentration. Biomass concentration increased exponentially afterwards in I-10 and O-10 up to steady state values of 1284±71 mg TSS/L and 1328±28 mg TSS/L, respectively. Similarly, biomass concentration in I-20 and O-20 increased up to steady state values of 720±16 and 620±79 mg TSS/L, respectively (Fig. 3). Indeed, the steady state biomass concentrations in systems supplied with 10 folds diluted PWW were ~2 times higher than those recorded in the photobioreactors fed with 20 folds diluted PWW (Table 1). On the other hand, the higher water evaporation rates in the outdoor photobioreactors resulted in slightly lower biomass productivities: 5.6, 3.1, 4.8 and 2.4 $g/m^2 \cdot d$ in I-10, I-20, O-10 and O-20, respectively. The lower biomass productivities recorded outdoors could be also explained by the pernicious effects on microbial metabolism caused by the high and fluctuating temperatures and irradiations. These biomass productivities were comparable to those reported by García et al. (2017b) under indoor conditions during the treatment of 15% diluted PWW in 3 L HRAPs operated at ≈ 27 days of HRT (5.8 to 7.8 $g/m^2 \cdot d$).

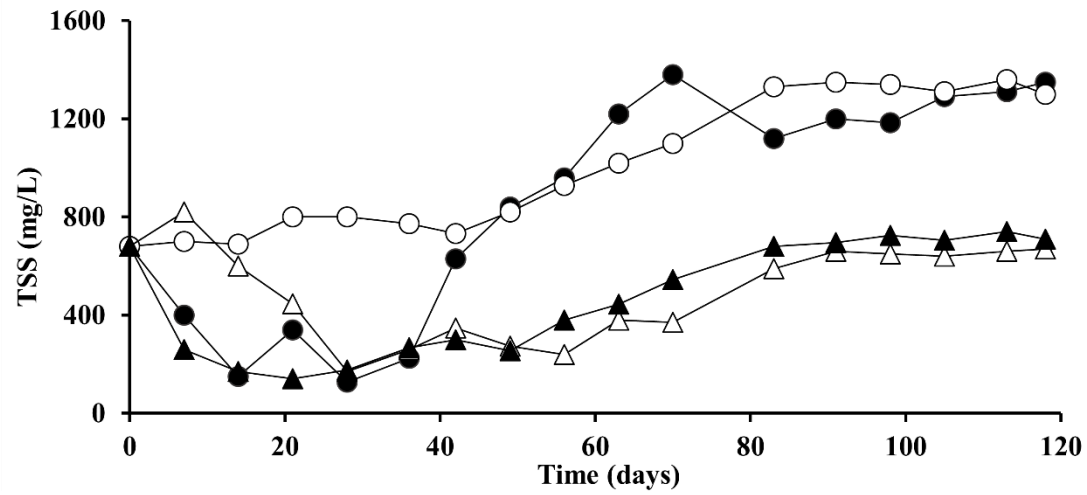


Figure 3. Time course of TSS concentration in I-10 (●), O-10 (○), I-20 (▲) and O-20 (Δ).

The C, N and P content of the biomass cultivated indoors averaged 49.3±0.6, 8.6±0.4 and 0.52±0.06% (which entailed a C/N/P of 100/17/1), respectively, and 46.0±0.02, 8.2±0.02 and 0.58±0.02% (C/N/P of 100/17/1), respectively, when cultivated outdoors. These elemental compositions were in agreement with those reported by Cabanelas et al. (2013), who observed a C, N and P content in the harvested biomass of ≈ 44, 7.5 and 0.5%, respectively, in a photobioreactor inoculated with *Chlorella vulgaris* and supplemented with CO_2 during the treatment of settled domestic wastewater.

3.4 Time course of the microalgae population structure

Chlorella vulgaris, which achieved a maximum cell concentration of $1.74 \cdot 10^9$ cells/L by day 92, represented the dominant photosynthetic species in I-10 throughout the entire experimental period. *Pseudanabaena* sp. was also identified in I-10 from day 92 onwards at concentrations of $\approx 0.30 \cdot 10^9$ cells/L (Fig. 4a). A similar microalgae population dynamics was recorded in I-20, with *C. vulgaris* representing the dominant species with maximum cell concentrations of $2.95 \cdot 10^9$ by days 42 and 70. However, *Pseudanabaena* sp. became dominant by day 120 with a concentration of $0.46 \cdot 10^9$ cells/L as a result of the gradual decrease in *C. vulgaris* population from day 70 (Fig. 4b). *C. vulgaris* was also dominant in the photobioreactors operated outdoors regardless of the PWW dilution applied. However, the maximum concentration of *C. vulgaris* in O-10 was recorded in the inoculum ($0.52 \cdot 10^9$ cells/L), with a gradual decrease afterwards. *Pseudanabaena* sp. was identified by days 105 and 120 at concentrations of

$0.15 \cdot 10^9$ and $0.08 \cdot 10^9$ cells/L, respectively, in O-10. In addition, *Aphanothece* sp. was also detected from day 70 to 120 in O-10, but at negligible concentrations (Fig. 4c). Finally, the maximum cell concentration of *C. vulgaris* in O-20 was $1.73 \cdot 10^9$ cells/L by day 25, with *Acutodesmus obliquus* (identified by day 25) and *Aphanothece* sp. (identified by days 56 and 72) detected at negligible concentrations, and *Pseudanabaena* sp. identified by day 92 at a concentration of $0.59 \cdot 10^9$ cells/L (Fig. 4d). The high tolerance of *C. vulgaris* to organic and heavy metals pollution likely supported the observed dominance of this microalga regardless of the operational and environmental conditions. Thus, *C. vulgaris* ranked 11/80 in the ranking of pollution-tolerant microalgae species published by Palmer (1969), while the *Chlorella* ranked 5/60 at a genus level. Process inoculation with *C. vulgaris* at a high concentration, along with the high tolerance of this microalga to organic pollution, guaranteed its long-term dominance and an effective PWW treatment. *Pseudanabaena* sp., which belongs to the order of *Oscillatoriales*, was also identified at relevant concentrations under steady state (Acinas et al., 2009). The tolerance of *Pseudanabaena* sp to organic pollution herein recorded was in agreement with the observations of García et al. (2017a), who identified *Pseudanabaena* sp. during the treatment of domestic wastewater in an enclosed photobioreactor at a HRT of 2 day, and by Serejo et al. (2015) during the treatment of digested vinasse in a 180 L HRAP. This study also suggested that the high and fluctuating temperatures and irradiations prevailing under outdoors operation resulted in both a reduced population of microalgae and cyanobacteria compared to indoors cultures, and in lower biomass productivities (Fig. 4a, 4b). Finally, the highest microalgae concentration recorded during the treatment of 20 times diluted PWW regardless of the environmental conditions was likely caused by the lower toxicity at increasing PWW dilutions.

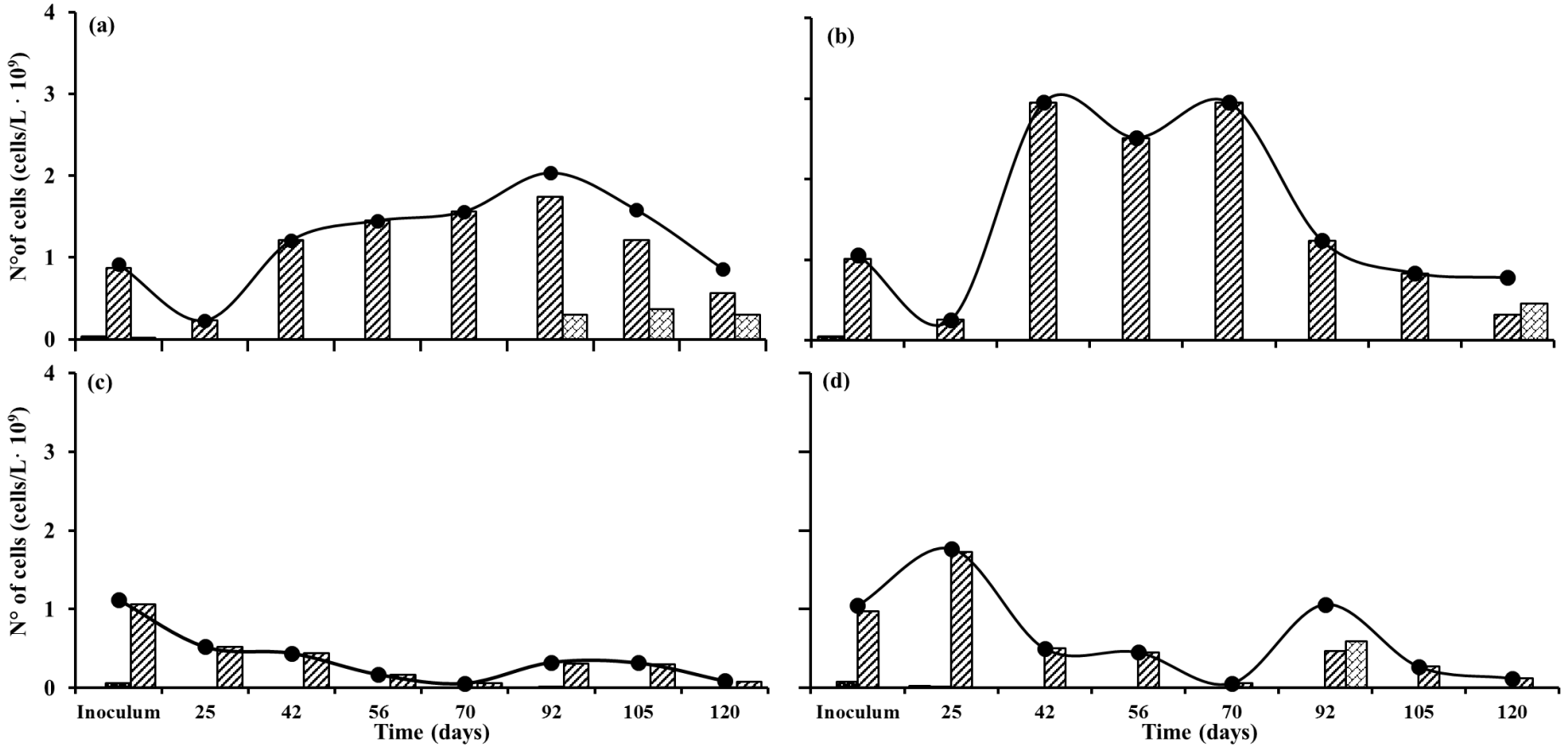


Figure 4. Time course of the microalgae population structure in I-10 (a), I-20 (b), O-10 (c) and O-20 (d). *Acutodesmus obliquus* ▩, *Aphanothece sp.* ■, *Chlorella vulgaris* ▨, *Pseudanabaena sp.* ▨, and total numbers of cells *(●)*.

3.5 Bacteria population structure

The DGGE analysis of the microbial communities present in the open photobioreactors revealed the occurrence of 4 phyla and 23 bands (Fig. 5). *Proteobacteria*, which is ubiquitous in the environment, was the dominant phylum (15 out of 23 bands sequenced) in the inoculum and in all photobioreactors (bands 1-15) (Fig. 5) (Shin et al., 2015). Despite not present in the inoculum, the phylum *Bacteroidetes* was identified under steady state in all photobioreactors (bands 16-20). The phylum *Firmicutes* was identified in the inoculum (bands 21 and 22) and in O-20, while the phylum *Cyanobacteria/Chloroplast* corresponded to band 23 in the inoculum and in I-10, I-20 and O-10 (Fig. 5). In this context, the open nature of the photobioreactors, along with the different environmental conditions and characteristics of the PWW fed, likely induced the enrichment of photobioreactor-specific bacterial populations different from the inoculum. Bacteria from the phyla *Proteobacteria*, *Bacteriodetes* and *Firmicutes* were likely the responsible for the biodegradation of organic matter in the photobioreactors. Thus, bacteria from the phylum *Proteobacteria* belonging to the genus *Psychrobacter* (I-10, I-20, O-10 and O-20), the class *Betaproteobacteria* (I-10, I-20, O-10 and O-20) and the genus *Thauera* (I-10, I-20 and O-10) have been identified in synthetic wastewater, swine effluents, anaerobic digesters treating feedstock from cheese manufacturing, wastewater from dye industry and anoxic biotrickling filters treating BTEX, which confirmed the capacity of these microorganisms to biodegrade the organic pollutants present in PWW (Akmirza et al., 2017; Lucas et al., 2013). Similarly, bacteria from the phylum *Bacteriodetes* have been identified during the anoxic removal of BTEX, Laboratory-scale partial nitrifying-ANAMMOX reactor and municipal wastewater treatment (Fig. 5) (Akmirza et al., 2017; Biswas and Turner, 2012). Finally, bacteria from the phylum *Firmicutes* (syntrophic microorganisms) were detected in a SBR reactor treating swine waste (Loureiro, 2008; Rivière et al., 2009).

The Shannon-Wiener diversity indexes (H) of the inoculum, I-10, I-20, O-10 and O-20 were 2.66, 2.69, 2.72, 2.63 and 2.17, respectively (Fig. 5). The photobioreactors operated in this study exhibited a relatively low-medium bacterial diversity ($H \approx 2.6$) likely due to the extreme environmental conditions

applied and to the high toxicity of the wastewater treated. The analysis of the similarity indexes (76.3% between I-10 and I-20 and 76.3% between O-10 and O-20) showed high similarities between the respective indoor and outdoor photobioreactors. On the other hand, low similarity indexes were recorded between I-10 and O-10 (14.2%) and I-20 and O-20 (41.6%). Thus, these results confirmed that temperature and irradiation under indoor and outdoor conditions can result in significantly different bacterial population structure. These results were in agreement with the findings reported by Ferrero et al. (2012), who observed that environmental parameters such as temperature or the impinging irradiation can play a more important role than organic matter and nutrients loading in the structure of the bacterial community.

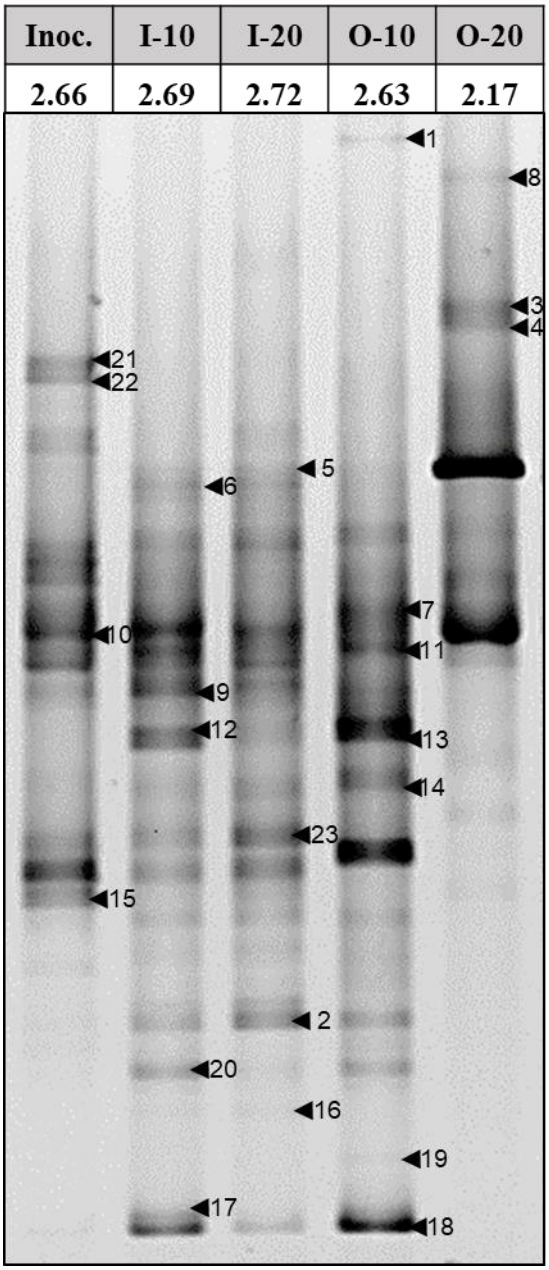


Figure 5. Bacterial DGGE profile of the microbial communities in the inoculum (Inoc.) and in the open algal-bacterial photobioreactors I-10, I-20, O-10 and O-20. Horizontal arrows and numbers indicate the most abundant bacterial communities. The name of the samples and the Shannon-Wiener diversity indexes (H) are also shown in the upper part of the gel profiles.

4. Conclusions

This work demonstrated for the first time that neither pollutant removal nor the structure of microalgae and bacterial communities under indoor conditions can be directly extrapolated to outdoors photobioreactors. Unexpectedly, the lowest PWW dilution always resulted in a superior PWW treatment performance. The dominance of *Chlorella vulgaris* in all photobioreactors regardless of the environmental conditions and PWW dilution confirmed the high pollution-tolerance of this species. The DGGE analysis revealed a high dominance of the *Proteobacteria* phylum in all photobioreactors, and the key influence of temperature and irradiation on the final bacterial population structure.

Acknowledgments

This research was supported by INIA, the FEDER EU program (RTA2013-00056-C03-02), the Regional Government of Castilla y León (Projects VA024U14 and UIC 71) and MINECO (Red Novedar). The financial support of the EU program ERASMUS MUNDUS EURICA and Universidad Nacional Autónoma de Nicaragua (UNAN-Managua) is also gratefully acknowledged.

References

1. Acinas, S.G., Haverkamp, T.H., Huisman, J., Stal, L.J., 2009. Phenotypic and genetic diversification of Pseudanabaena spp. (cyanobacteria). The ISME Journal 378, 31–46. doi:10.1038/ismej.2008.78

2. Aguirre, P., Álvarez, E., Ferrer, I., García, J., 2011. Treatment of piggery wastewater in experimental high rate algal ponds. Rev Latinoam Biotecnol Amb Algal 2, 57–66.

3. Akmirza, I., Pascual, C., Carvajal, A., Pérez, R., Muñoz, R., Lebrero, R., 2017. Science of the Total Environment Anoxic biodegradation of BTEX in a biotrickling fi lter 588, 457–465. doi:10.1016/j.scitotenv.2017.02.130

4. Alcántara, C., Domínguez, J.M., García, D., Blanco, S., Pérez, R., García-Encina, P.A., Muñoz, R., 2015. Evaluation of wastewater treatment in a novel anoxic-aerobic algal-bacterial photobioreactor with biomass recycling through carbon and nitrogen mass balances. Bioresource Technology 191, 173–186. doi:10.1016/j.biortech.2015.04.125

5. Anbalagan, A., Schwede, S., Lindberg, C.-F., Nehrenheim, E., 2016. Influence of hydraulic retention time on indigenous microalgae and activated sludge process. Water Research 91, 277–284. doi:10.1016/j.watres.2016.01.027

6. APHA, 2005. Standards Methods for the Examination of Water and Wastewater, 21 st. ed. American Public Health Association, American Water Works Association, Water Enviroment Federation, Washington,D.C.

7. Bastos, R.G., Queiroz, Zepka, L.Q., Volpato, G., García, Jacob-Lopes, 2009. COD Removal of Parboilized Rice Wastewater By

Cyanobacteria Aphanothece Microscopica Nägeli. BioEng, Campinas 3, 245–250.

8. Biswas, K., Turner, S.J., 2012. Microbial Community Composition and Dynamics of Moving Bed Biofilm Reactor Systems Treating Municipal Sewage 855–864. doi:10.1128/AEM.06570-11

9. Cabanelas, I.T.D., Ruiz, J., Arbib, Z., Chinalia, F.A., Garrido-Pérez, C., Rogalla, F., Nascimento, I.A., Perales, J.A., 2013. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresource Technology 131, 429–436. doi:10.1016/j.biortech.2012.12.152

10. Cheah, W.Y., Ling, T.C., Show, P.L., Juan, J.C., Chang, J.S., Lee, D.J., 2016. Cultivation in wastewaters for energy: A microalgae platform. Applied Energy 179, 609–625. doi:10.1016/j.apenergy.2016.07.015

11. Council Directive, 1999. Council Directive 1999/31/EC on the landfill. Official Journal of the European Communities L182/1-19. doi:10.1039/ap9842100196

12. Dassey, A.J., Theegala, C.S., 2013. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresource Technology 128, 241–245. doi:10.1016/j.biortech.2012.10.061

13. De Godos, I., Blanco, S., García-Encina, P.A., Becares, E., Muñoz, R., 2009. Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates. Bioresource Technology 100, 4332–4339. doi:10.1016/j.biortech.2009.04.016

14. EU, 2015. http://ec.europa.eu [WWW Document]. URL http://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_animals (accessed 1.10.17).

15. European Commisssion, 2008. Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain directives. Official Journal of the European Union L13, 3–30. doi:2008/98/EC.; 32008L0098

16. Ferrero, E.M., de Godos, I., Rodríguez, E.M., García-Encina, P.A., Muñoz, R., Bécares, E., 2012. Molecular characterization of bacterial communities in algal-bacterial photobioreactors treating piggery wastewaters. Ecological Engineering 40, 121–130. doi:10.1016/j.ecoleng.2011.10.001

17. Franchino, M., Tigini, V., Varese, G.C., Mussat Sartor, R., Bona, F., 2016. Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. Science of The Total Environment 569, 40–45. doi:10.1016/j.scitotenv.2016.06.100

18. Frutos, O.D., Arvelo, I.A., Pérez, R., Quijano, G., Muñoz, R., 2015. Continuous nitrous oxide abatement in a novel denitrifying off-gas bioscrubber. Applied microbiology and biotechnology 99, 3695–706. doi:10.1007/s00253-014-6329-8

19. García, D., Alcántara, C., Blanco, S., Pérez, R., Bolado, S., Muñoz, R., 2017a. Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading. Chemical Engineering Journal 313, 424–434. doi:10.1016/j.cej.2016.12.054

20. García, D., Posadas, E., Blanco, S., Acién, G., Garcia-Encina, P., Bolado, S., Muñoz, R., 2017b. Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors. Bioresource Technology.

doi:10.1016/j.biortech.2017.06.079

21. González, C., Marciniak, J., Villaverde, S., García-Encina, P.A., Muñoz, R., 2008. Microalgae-based processes for the biodegradation of pretreated piggery wastewaters. Applied Microbiology and Biotechnology 80, 891–898. doi:10.1007/s00253-008-1571-6

22. Häne, B.G., Jäger, K., Drexler, H.G., 1993. The Pearson product-moment correlation coefficient is better suited for identification of DNA fingerprint profiles than band matching algorithms. Electrophoresis 14, 967–972. doi:10.1002/elps.11501401154

23. Javanbakht, V., Alavi, S.A., Zilouei, H., 2014. Mechanisms of heavy metal removal using microorganisms as biosorbent. Water Science and Technology 69, 1775–1787. doi:10.2166/wst.2013.718

24. Kaplan, D., Cristiaen, D., Shoshana, A., 1987. Chelating Properties of Extracellular Polysaccharides from Chlorella spp. Applied and Environmental Microbiology. 53, 2953–2956.

25. Loureiro, M., 2008. Improving stability of anaerobic digesters for animal waste treatment.

26. Lucas, J.., García-Villaraco, A., Ramos, B., García-Cristobal, E., Algar, E., Gutierrez-Mañero, J., 2013. Structural and functional study in the rhizosphere of Oryza sativa L . plants growing under biotic and abiotic stress. doi:10.1111/jam.12225

27. MacDonald, G.M., 2003. Space , Time , and Life. John Wiley & Sons, Inc, New York, USA.

28. MAGRAMA, 2015. Censo y producción: Producción de porcino ibérico [WWW Document]. URL http://www.magrama.gob.es/es/ganaderia/temas/produccion-y-mercados-ganaderos/sectores-ganaderos/porcino/ (accessed 10.18.16).

29. McGinnis, S., Madden, T.L., 2004. BLAST: At the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Research 32, 20–25. doi:10.1093/nar/gkh435

30. Metcalf, E., Eddy, H., 2003. Wastewater engineering: treatment and reuse. Tata McGraw-Hill Publishing Company Limited, 4th edition. New Delhi, India. doi:10.1016/0309-1708(80)90067-6

31. Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: A review. Water research 40, 2799–2815. doi:10.1016/j.watres.2006.06.011

32. Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R.I., Ludwig, W., Backhaus, H., 1996. Sequence heterogeneities of genes encoding 16S rRNA in Paenibacillus polymyxy detected by temperature gradient gel electrophoresis. Journal of Bacteriology 178, 5636–5643.

33. Oswald, W.J., Gotaas, H.B., Golueke, C.G., Kellen, W.R., Gloyna, E.F., Hermann, E.R., 1957. Algae in Waste Treatment [with Discussion]. Water Environment Federation 29, 437–457.

34. Palmer, C.M., 1969. A composite rating of algae tolerating organic pollution. Journal of Phycology 5, 78–82. doi:10.1111/j.1529-8817.1969.tb02581.x

35. 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. Bioresource Technology 139, 50–58. doi:10.1016/j.biortech.2013.04.008

36. Posadas, E., Marín, D., Blanco, S., Lebrero, R., Muñoz, R., 2017. Simultaneous biogas upgrading and centrate treatment in an

outdoors pilot scale high rate algal pond. Bioresource Technology 232, 133–141. doi:10.1016/j.biortech.2017.01.071

37. Posadas, E., Morales, M.D.M., Gomez, C., Acién, F.G., Muñoz, R., 2015. Influence of pH and CO2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chemical Engineering Journal 265, 239–248. doi:10.1016/j.cej.2014.12.059

38. Rivière, D., Desvignes, V., Pelletier, E., Chaussonnerie, S., Guermazi, S., Weissenbach, J., Li, T., Camacho, P., Sghir, A., 2009. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. The ISME Journal 3, 700–714. doi:10.1038/ismej.2009.2

39. Roest, K., Heilig, H.G.H.J., Smidt, H., de Vos, W.M., Stams, A.J.M., Akkermans, A.D.L., 2005. Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater. Systematic and applied microbiology 28, 175–185. doi:10.1016/j.syapm.2004.10.006

40. Serejo, M.L., Posadas, E., Boncz, M.A., Blanco, S., García-Encina, P., Muñoz, R., 2015. Influence of Biogas Flow Rate on Biomass Composition During the Optimization of Biogas Upgrading in Microalgal-Bacterial Processes. Environmental Science & Technology 3228–3236. doi:10.1021/es5056116

41. Shin, N.-R., Whon, T.W., Bae, J.-W., 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends in biotechnology 33, 496–503. doi:10.1016/j.tibtech.2015.06.011

42. Sournia, A., 1978. Phytoplankton manual. UNESCO, Paris.

43. statista, 2016. Global pork production in 2016, by country [WWW Document]. URL https://www.statista.com

44. Tigini, V., Franchino, M., Bona, F., Varese, G.C., 2016. Is digestate safe? A study on its ecotoxicity and environmental risk on a pig manure. The Science of the total environment 551–552, 127–132. doi:10.1016/j.scitotenv.2016.02.004

45. Wang, H.-F., Zhu, W.-Y., Yao, W., Liu, J.-X., 2007. DGGE and 16S rDNA sequencing analysis of bacterial communities in colon content and feces of pigs fed whole crop rice. Anaerobe 13, 127–133. doi:10.1016/j.anaerobe.2007.03.001

46. Wang, M., Sahu, A.K., Rusten, B., Park, C., 2013. Anaerobic co-digestion of microalgae Chlorella sp. and waste activated sludge. Bioresource technology 142, 585–90. doi:10.1016/j.biortech.2013.05.096

SUPPLEMENTARY MATERIAL

Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions

Dimas García[1,2], Esther Posadas[1], Carlos Grajeda[1], Saúl Blanco[3], Sonia Martínez-Páramo[1], Gabriel Acién[4], Pedro García-Encina[1], Silvia Bolado[1], Raúl Muñoz[1]*

[1]Department of Chemical Engineering and Environmental Technology, School of Industrial Engineerings, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

[2]Centro para la Investigación de los Recursos Acuáticos de Nicaragua, CIRA/UNAN-Managua, Apdo. Postal 4598, Nicaragua

[3]The Institute of the Environment, La Serna 58 - 24007 León, Spain

[4]Department of Chemical Engineering, University of Almeria, Cañada San Urbano, s/n, 04120 Almeria, Spain

*corresponding author: mutora@iq.uva.es

CONTENT

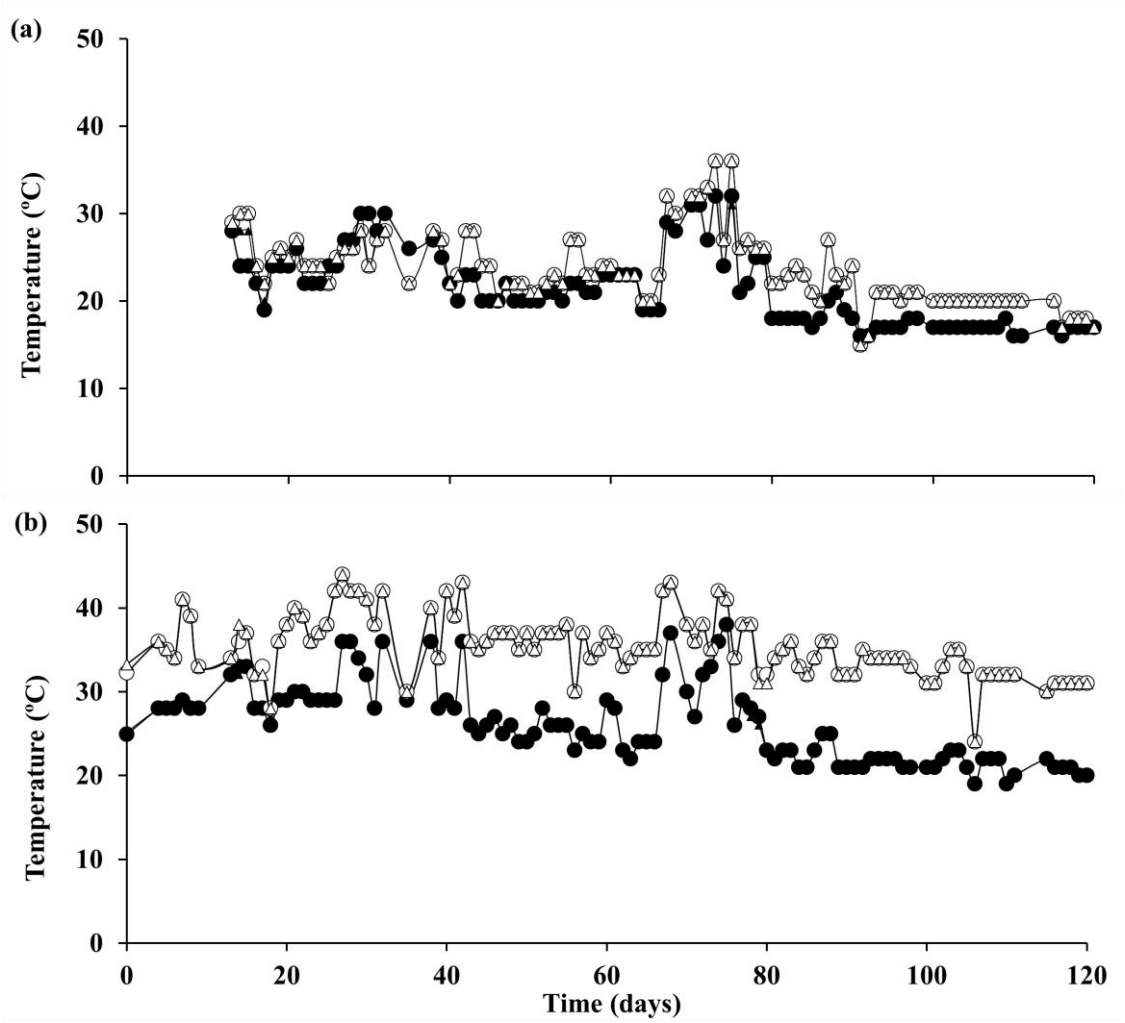


Figure S1. Time course of the temperature at 11h00 (a) and 17h00 (b) in I-10 (●), I-20 (▲), O-10 (○) and O-20 (Δ) throughout the experimental period.

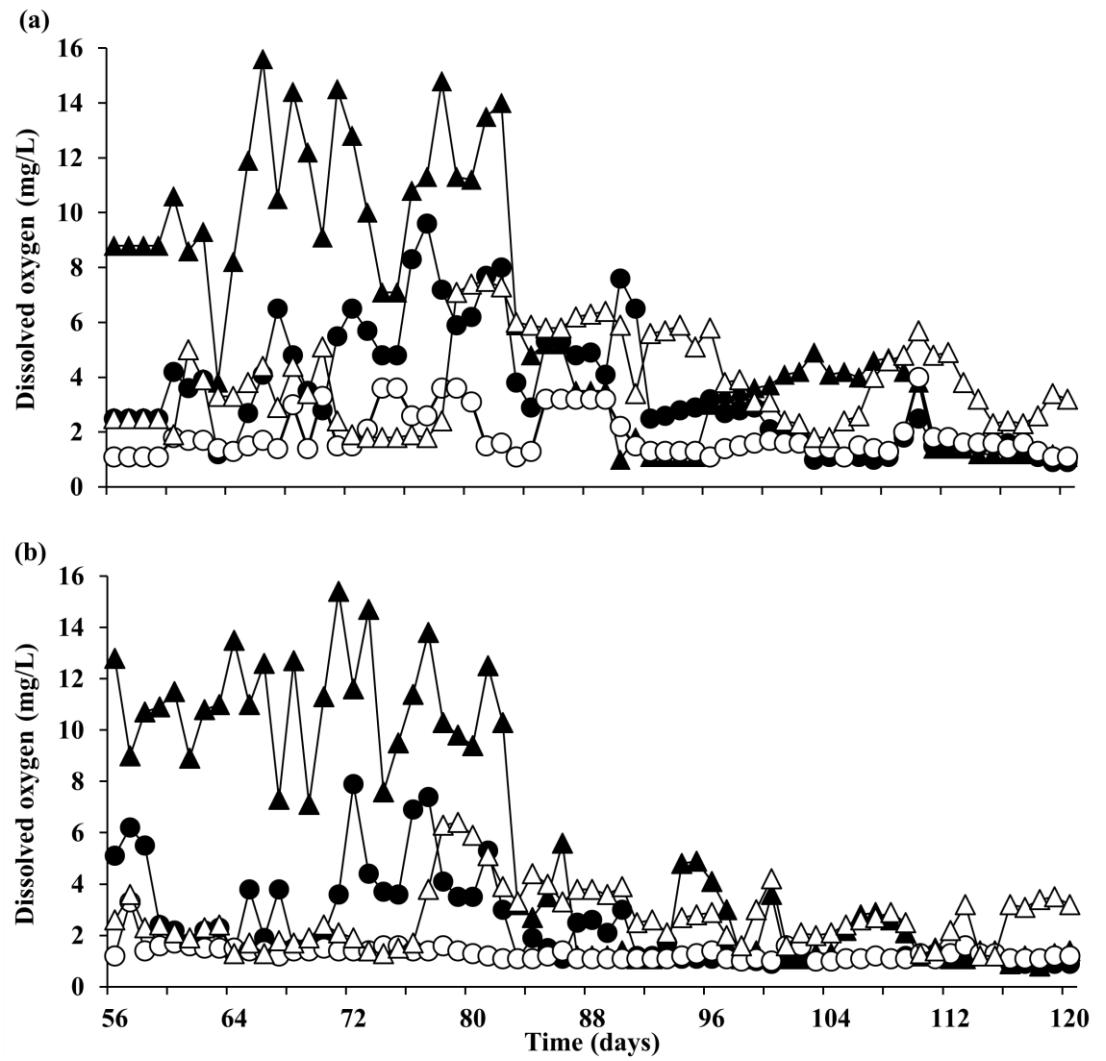


Figure S2. Time course of the dissolved oxygen concentration at 11h00 (a) and 17h00 (b) in I-10 (●), I-20 (▲), O-10 (○) and O-20 (Δ) throughout the experimental period.

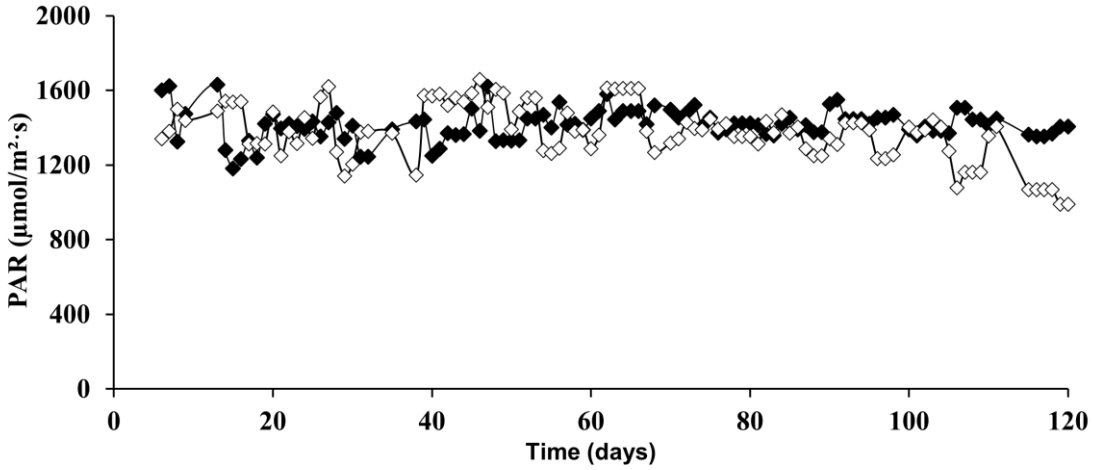


Figure S3. Time course of the average PAR under indoor (■) and outdoor (□) conditions.

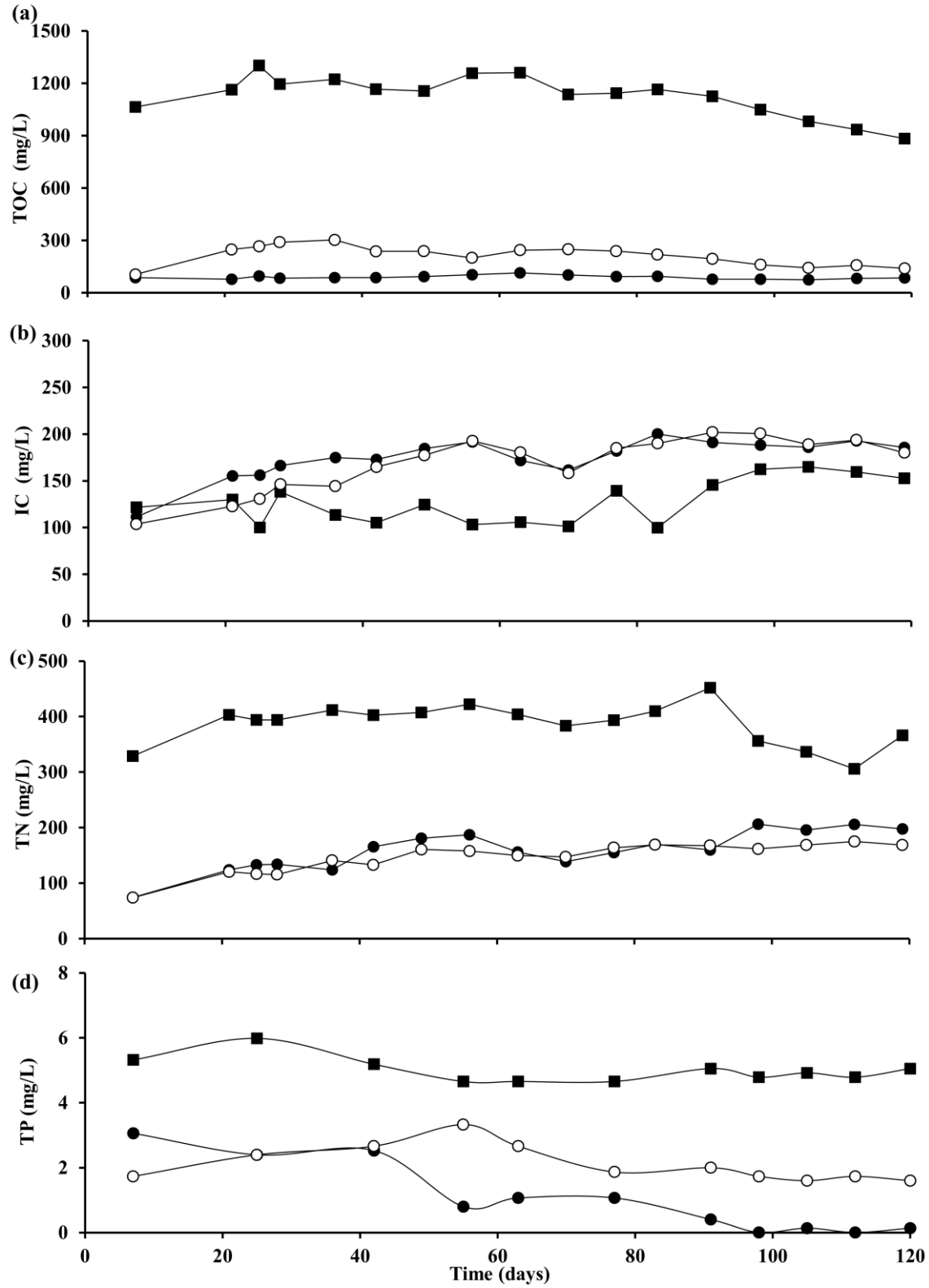


Figure S4. Time course of the concentration of TOC (a), IC (b) TN (c) and TP (d) in the 10 fold diluted PWW (■), I-10 (●) and O-10 (○).

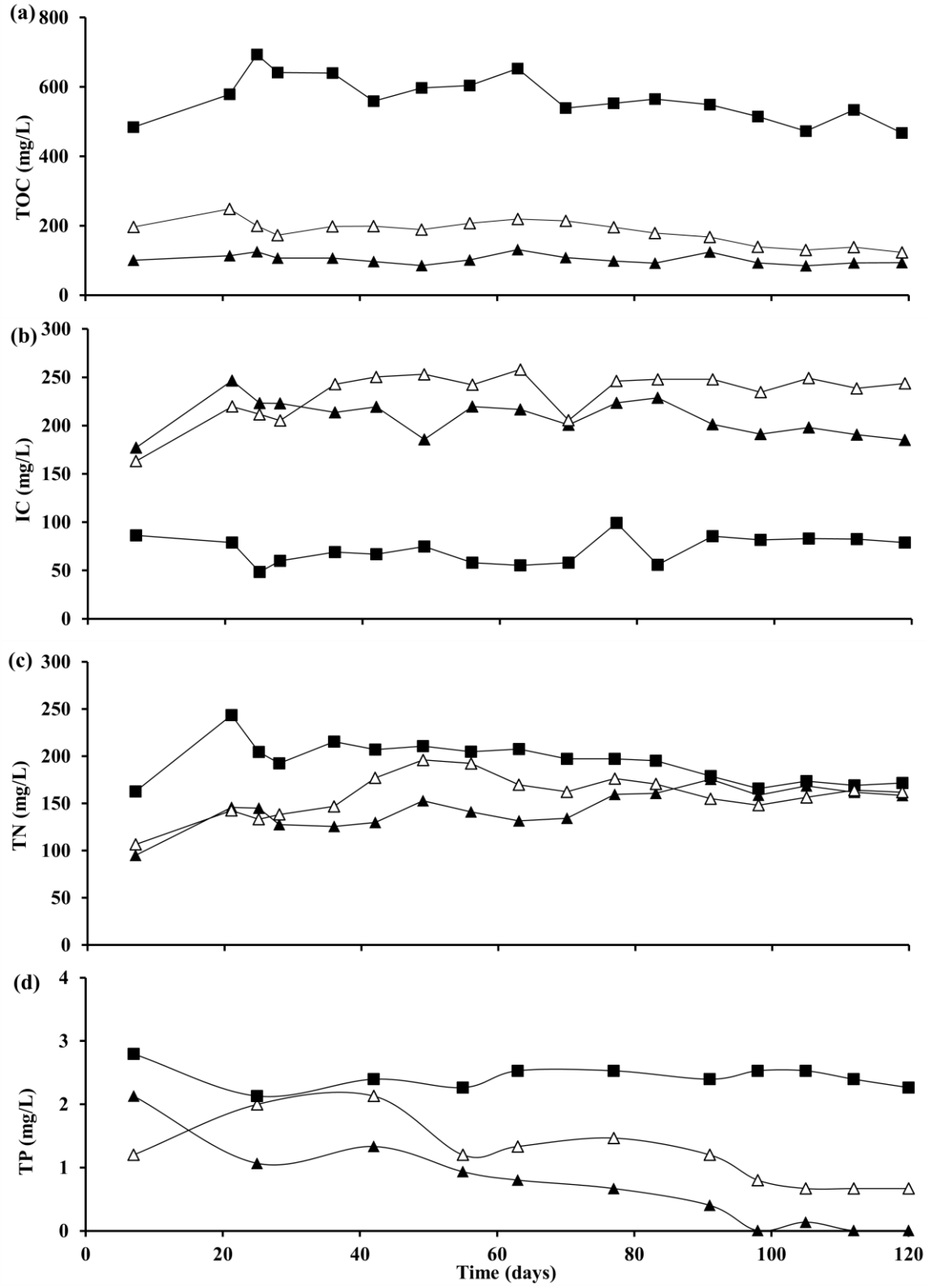


Figure S5. Time course of the concentration of TOC (a), IC (b) TN (c) and TP (d) in the 20 fold diluted PWW (■), I-20 (▲) and O-20 (Δ).

Table S1. Operational parameters and photobioreactor dimensions under indoor conditions.

HRT (d)	**Q*feed* (L/d)**	**Q*eff* (L/d)**	**Evaporation %**	**V PBR (L)**	**Height PBR (cm)**	**Area PBR (m^2)**
27	0.113	0.082	27	3.0	15.8	0.019

PBR: Photobioreactor

Table S2. Mass balance calculations of PWW treatment under steady state in the indoor photobioreactors**.**

Parameter	PWW (×10)	PWW (×20)	I-10	I-20
TOC (mg/L)	963	497	80	91
TOC (mg/d) in			108.3	55.9
TOC (mg/d) out			6.6	7.5
% TOC RE			94	87
IC (mg/L)	160	82	188	191
IC (mg/d) in			18.0	9.2
IC (mg/d) out			15.4	15.7
% IC RE			14	-70
TN (mg/L)	341	170	201	162
TN (mg/d) in			38.4	19.1
TN (mg/d) out			16.5	13.3
% TN RE			57	30
Nitrite (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5
Nitrate (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5
TP (mg/L)	4.9	2.5	0.07	0.04
TP (mg/d) in			0.55	0.28
TP (mg/d) out			0.01	0.00
% TP RE			99	99
Zinc (mg/L)	0.67	0.37	0.16	0.15
Zn (mg/d) in			0.08	0.04
Zn (mg/d) out			0.01	0.01
% Zn RE			83	70
TSS (mg/L)	291	156	1284	720
Productivity ($g/m^2/d$)			5.55	3.11
C %			48.82	49.72
C (mg/d) biomass			51.48	29.40
% Carbon recovered			49	70
N %			8.88	8.37
N (mg/d) biomass			9.36	4.95
% Nitrogen recovered			43	85
P%			0.56	0.47
P (mg/d) biomass			0.59	0.28
% Phosphorus recovered			100	100

Table S3. Operational parameters and photobioreactor dimensions under outdoors conditions. **PBR**: Photobioreactor

HRT (d)	**Q*feed* (L/d)**	**Q*eff* (L/d)**	**Evaporation %**	**V PBR (L)**	**Height PBR (cm)**	**Area PBR (m^2)**
25	0.122	0.068	44	3.0	15.8	0.019

Table S4. Mass balance calculations of PWW treatment under steady state in the outdoor photobioreactors**.**

Parameter	PWW (×10)	PWW (×20)	O-10	O-20
TOC (mg/L)	963	497	150	133
TOC (mg/d) in			117	61
TOC (mg/d) out			10.2	9.1
% TOC RE			91	85
IC (mg/L)	160	82	191	241
IC (mg/d) in			20	10
IC (mg/d) out			13	16
% IC RE			33	-65
TN (mg/L)	341	170	168	158
TN (mg/d) in			41.6	20.7
TN (mg/d) out			11.5	10.8
% TN RE			72	48
Nitrite (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5
Nitrate (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5
TP (mg/L)	4.9	2.5	1.67	0.7
TP (mg/d) in			0.60	0.31
TP (mg/d) out			0.114	0.048
% TP RE			81	84
Zinc (mg/L)	0.67	0.37	0.35	0.32
Zn (mg/d) in			0.08	0.05
Zn (mg/d) out			0.02	0.02
% Zn RE			71	52
TSS (mg/L)	291	156	1328	655
Productivity ($g/m^2/d$)			4.78	2.36
C %			46.0	46.0
C (mg/d) biomass			41.7	20.6
% Carbon recovered			37	46
N %			8.19	8.22
N (mg/d) biomass			7.43	3.68
% Nitrogen recovered			25	37
P%			0.57	0.59
P (mg/d) biomass			0.52	0.26
% Phosphorus recovered			100	100

Table S5. RDP classification of the DGGE bands sequenced and corresponding matches (BLASTN) using the NCBI database with indication of the similarity percentages and sources of origin. The presence/absence of each band in each sample, together with its intensity, are also shown.

Taxonomic placement (50% confidence level)	Band nº	Inoc.	I-10	I-20	O-10	O-20	Closest relatives in Blast Name (Accession number)	Similarity (%)	Source of origin
Phylum Proteobacteria	1	-	-	-	X	-	Uncultured bacterium (JQ320097)	87%	Soil polluted with BDE209 and Cd
Class *Gammaproteobacteria*	2	-	XX	XX	XX	-	Uncultured bacterium (JQ300186)	90%	Soil
Order *Pseudomonadales*									
Family *Moraxellaceae*									
Genus *Psychrobacter*	3	-	-	-	-	XX	Uncultured *proteobacterium* (JQ218906)	96%	Marine macro-alga
							Psychrobacter sp. Bsw21512 (GQ358937)	96%	Seawater
							Uncultured bacterium (JF332609)	96%	Duodenal biopsy
	4	-	-	-	-	XX	Uncultured *Psychrobacter* sp. (JQ999390)	97%	Lake Vostok accretion ice (Antarctica)
							Uncultured bacterium (JF332609)	97%	Duodenal biopsy
							Psychrobacter sp. (KY406049)	96%	Soil sample from penguin breeding colony
	5	-	XX	XX	X	XXX	*Psychrobacter piscatorii* (NR_112807)	99%	Wastewater
							Psychrobacter psychrophilus (DQ337513)	99%	Swine effluent holding pit
	6	-	XX	XX	X	XXX	*Psychrobacter* sp. Mixed culture X14-2 (KR029412)	99%	Bioaerosol emitted from wastewater treatment plant
							Uncultured bacterium (JF332609)	99%	Duodenal biopsy
							Uncultured bacterium (KR514346)	99%	Bovine reproductive tract
	7	-	-	-	XXX	-	Uncultured *Psychrobacter*	100%	Lake Vostok accretion ice

Taxonomic placement (50% confidence level)	Band nº	Inoc.	I-10	I-20	O-10	O-20	Closest relatives in Blast Name (Accession number)	Similarity (%)	Source of origin
							sp. (JQ999390)		(Antarctica)
							Psychrobacter sp. Mixed culture X14-2 (KR029412)	100%	Bioaerosol emitted from wastewater treatment plant
							Psychrobacter sp. KHH8 (KT368953)	100%	-
	8	-	-	-	-	XX	*Psychrobacter* sp. Mixed culture X14-2 (KR029412)	97%	Bioaerosol emitted from wastewater treatment plant
							Uncultured Psychrobacter sp. (JQ999390)	97%	Lake Vostok accretion ice (Antarctica)
Class *Betaproteobacteria*	9	XX	XXX	XX	XXX	-	Uncultured bacterium (GU390196)	86%	Anaerobic digester treating feedstock
	10	XXX	XXX	XXX	-	XXX	*Acinetobacter* sp. HPC497 (AY854128)	89%	Wastewater from dye industry
	11	XXX	XXX	-	XXX	-	Uncultured bacterium (KU991981)	87%	Anoxic removal of BTEX compounds
Order *Rhodocyclales*									
Family *Rhodocyclaceae*									
Genus *Thauera*	12	-	XX	XX	XXX	-	Uncultured bacterium (HG380609)	97%	Wastewater
							Uncultured beta *proteobacterium* (AF450463)	97%	Full-scale aerated-anoxic wastewater treatment plant
							Uncultured *Thauera* sp. (KX914731)	97%	Activated sludge
	13	-	XX	-	XXX	-	*Thauera* sp. (MF155554)	98%	Wastewater treatment plant
							Uncultured bacterium (HG380609)	98%	Wastewater
							Uncultured beta *proteobacterium* (AF450463)	98%	Full-scale aerated-anoxic wastewater treatment plant
Class *Alphaproteobacteria*	14	X	XX	XX	XX	-	Uncultured bacterium (KT200337)	86%	Algal-bacterial biomass from an air-lift bioreactor treating toluene,

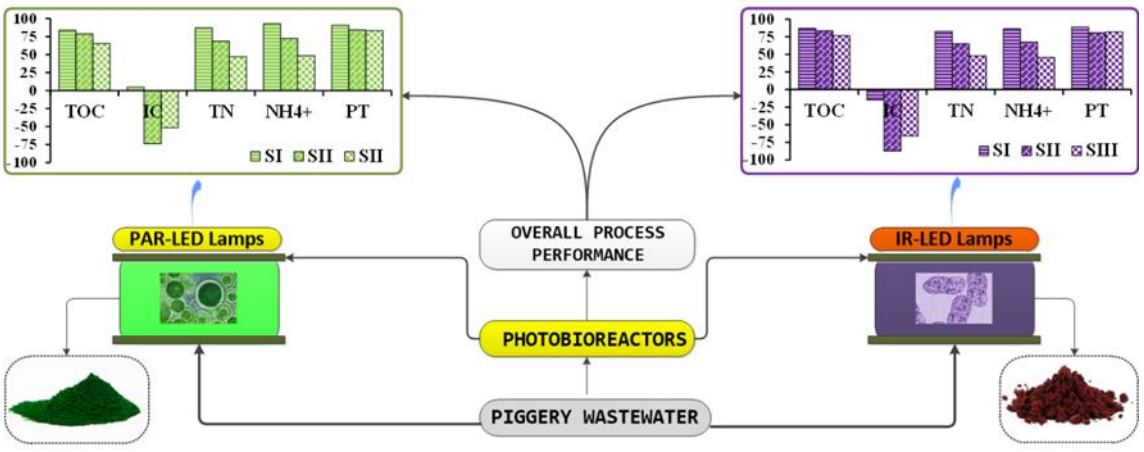
Taxonomic placement (50% confidence level)	**Band nº**	**Inoc.**	**I-10**	**I-20**	**O-10**	**O-20**	**Closest relatives in Blast Name (Accession number)**	**Similarity (%)**	**Source of origin**
									inoculated with activated sludge, *Pseudomonas putida* and *Chlorella Sorokiniana*
Order *Rhizobiales*	15	XX	-	-	-	X	Iron-reducing bacterium enrichment culture clone fec_1_F2 (FJ802355)	89%	Danube River sediment
Phylum *Bacteroidetes*	16	-	-	X	-	-	Uncultured bacterium (KU991981)	90%	Anoxic removal of BTEX compounds
							Uncultured bacterium (KU650792)	90%	Anaerobic full-scale reactors
							Uncultured bacterium (JN087868)	90%	Nitrifying bioreactor under inorganic carbon limitation
	17	-	XX	-	XX	-	Uncultured bacterium (HQ640531)	98%	Laboratory-scale partial nitrifying-ANAMMOX reactor
							Uncultured bacterium (KU650792)	98%	Anaerobic full-scale reactors
							Uncultured bacterium (JN087868)	98%	Nitrifying bioreactor under inorganic carbon limitation
	18	-	XXX	XX	XXX	-	Uncultured bacterium (HQ640531)	98%	Laboratory-scale partial nitrifying-ANAMMOX reactor
							Uncultured bacterium (KU650792)	98%	Anaerobic full-scale reactors
							Uncultured bacterium (JN087868)	98%	Nitrifying bioreactor under inorganic carbon limitation
Class *Cytophagia*									
Order *Cytophagales*	19	-	-	-	X	-	Uncultured bacterium (HQ640531)	100%	Laboratory-scale partial nitrifying-ANAMMOX reactor
							Uncultured bacterium (KU650792)	99%	Anaerobic full-scale reactors
Class *Sphingobacteria*									
Order *Sphingobacteriales*	20	-	XX	X	XX	-	Uncultured bacterium (KU650792)	99%	Anaerobic full-scale reactors

Taxonomic placement (50% confidence level)	**Band nº**	**Inoc.**	**I-10**	**I-20**	**O-10**	**O-20**	**Closest relatives in Blast Name (Accession number)**	**Similarity (%)**	**Source of origin**
							Uncultured bacterium (JN087868)	99%	Nitrifying bioreactor under inorganic carbon limitation
							Uncultured bacterium (KU991981)	99%	Anoxic removal of BTEX compounds
Phylum *Firmicutes*									
Class *Clostridia*									
Order Clostridiales	21	XX	-	-	-	-	Uncultured bacterium (GQ132773)	86%	SBR reactor treating swine waste; reactor 2, day 809; temperature: 35 deg C; ammonia: 1,800 mg N/L; solids loading rate: 2.2 g VS/L/day
	22	XX	-	-	-	XX	Uncultured bacterium (KP797907)	87%	Microalgae from HRAP treating diluted vinasse with wastewater treatment plant activated sludge
Phylum *Cyanobacteria/Chloroplast*									
Class *Chloroplast*									
Family *Chloroplast*									
Genus *Chlorophyta*	23	XX	XX	XXX	XXX	-	Uncultured bacterium (KT200344)	98%	Algal-bacterial biomass from an air-lift bioreactor treating toluene, inoculated with activated sludge, *Pseudomonas putida* and *Chlorella Sorokiniana*
							Plastid *Chlorella* sp. UMPCCC 1110 (KM218897)	98%	Water
							Uncultured *Chlorella* (KC994689)	96%	Microalgae photobioreactor

Chapter 4

A systematic comparison of the potential of a microalgal-bacterial consortium and purple phototrophic bacteria for the continuous treatment of piggery wastewater

García, D., de Godos, I., Dominguez, C., Turiel, S., Bolado, S., Muñoz, R. 2018. Submitted for puplication to Bioresource Technology. 27 August 2018



A systematic comparison of the potential of a microalgal-bacterial consortium and purple phototrophic bacteria for the continuous treatment of piggery wastewater

Dimas García[1,2,3], Ignacio de Godos[1,2,5], Christian Domínguez[1], Sara Turiel[4], Silvia Bolado[1,2], Raúl Muñoz[1,2]*

[1]Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

[2]Institute of Sustainable Processes, Dr. Mergelina, s/n, 47011, Valladolid, Spain

[3]Centro para la Investigación de los Recursos Acuáticos de Nicaragua, CIRA/UNAN-Managua, Apdo. Postal 4598, Nicaragua

[4]The Institute of the Environment, La Serna 58 – 24007, León, Spain

[5]School of Forestry, Agronomic and Bioenergy Industry Engineering (EIFAB), Valladolid University, Campus Duques de Soria, Soria, Spain.

*corresponding author: mutora@iq.uva.es

Abstract

This study evaluated the performance of two open photobioreactors operated with a consortium of microalgae-bacteria (PBR-AB) and purple photosynthetic bacteria (PBR-PPB) during the continuous treatment of diluted (5%) piggery wastewater (PWW) at multiple hydraulic retention times (HRT). At a HRT of 10.6 days, PBR-AB supported the highest removal efficiencies of nitrogen, phosphorous and zinc (87±2, 91±3 and 98±1%), while the highest organic carbon removals were achieved in PBR-PPB (87±4%). The decrease in HRT from 10.6, to 7.6 and 4.1 day caused a gradual deterioration in organic material and nitrogen removal, but did not influence the removal of phosphorus and Zn. The decrease in HRT caused a severe wash-out of microalgae in PBR-AB and played a key role on the structure of bacterial population in both photobioreactors. In addition, batch biodegradation tests at multiple PWW dilutions (5, 10 and 15%) confirmed the slightly better performance of algal-bacterial systems regardless of PWW dilution.

Keywords
Algal-bacterial processes;
Photobioreactor;
Photosynthetic biodegradation;
PPB;
Swine manure.

1. Introduction

The large volumes of wastewater yearly generated from domestic, industrial and agricultural activities demand a rapid and cost-effective wastewater treatment prior discharge into natural water bodies. More specifically, an insufficient treatment of agroindustrial effluents, which rank among the highest strengths wastewaters, can cause severe episodes of eutrophication in surface waters and pollution of groundwater (Mateo-sagasta and Burke, 2012). Only in the European Union, 215-430 Mm^3 of piggery wastewaters (PWW) with [COD] > 50 g L^{-1} or [TN] > 5 g L^{-1} are annually generated (García et al., 2018; statista, 2018). Conventional agricultural wastewater treatment (WWT) technologies (e.g. activated sludge, trickling filters) are highly energy intensive and entail a significant loss of valuable nutrients. In this context, photosynthetic treatments have emerged as a cost-effective alternative to conventional WWT based on their potential to support a superior nutrients and carbon recovery from agricultural wastewaters.

Photosynthetic WWT has been traditionally based on the cultivation of microalgae, which produce O_2 and assimilate nutrients using the visible spectrum of sunlight as energy source, in symbiosis with heterotrophic and nitrifying bacteria. More specifically, microalgal-bacterial consortia can support an efficient removal of organic matter, nutrients, heavy metals and pathogens as a result of their dual autotrophic and heterotrophic metabolism (Rittmann and McCarty, 2012). This symbiosis results in a low energy consumption and carbon footprint since the CO_2 generated during organic matter oxidation is photosynthetically fixed (Cheah et al., 2016; Dassey and Theegala, 2013). Algal-bacterial processes have been successfully tested for the treatment of domestic wastewater (García et al., 2017a; Oswald et al., 1957), centrates (Posadas et al., 2017), vinasse (Serejo et al., 2015), digested livestock effluents (Franchino et al., 2016; Tigini et al., 2016), parboiled rice wastewater (Bastos et al., 2009) and PWW (de Godos et al., 2010; García et al., 2018, 2017b).

On the other hand, purple phototrophic bacteria (PPB) can also use solar radiation (the infrared spectrum) as energy source during anoxygenic photosynthesis, which requires electron donors such as organic matter and nutrients to built-up PPB biomass (Bertling et al., 2006). PPB can support high rates of organic matter and nutrient assimilation and exhibit a high tolerance towards wastewater toxicity. Furthermore, there is an increasing interest in PPB-based WWT since PPB are able to synthesize polyhydroxybutyrates (PHB) and polyphosphates, and possess a more versatile metabolism than microalgae (Hülsen et al., 2014). PPB have been recently used to treat domestic wastewaters (Hülsen et al., 2016a, 2016b, 2014; Zhang et al., 2003), PWW (Myung et al., 2004), rubber sheet wastewater (Kantachote et al., 2005), pharmaceutical wastewater (Madukasi et al., 2010) and fish industry effluent (de Lima et al., 2011) with promising results. However, while microalgae-based WWT has been evaluated both indoors and outdoors in open and enclosed photobioreactors from lab scale to industrial facilities (Craggs et al., 2012; de Godos et al., 2016), PPB-based WWT has been only evaluated indoors under lab scale conditions. In this context, there is a lack of comparative studies systematically assessing the treatment capacity of both phototrophic microorganisms.

This work aimed at systematically evaluating the performance of open algal-bacterial and PPB photobioreactors for the indoor treatment of PWW under artificial illumination. The influence of the hydraulic retention time (HRT) on the removal of carbon, nitrogen, phosphorous and heavy metals, and on the structure of the algal-bacterial and PPB population was investigated. In addition, the influence of PWW dilution on the biodegradation performance of an algal-bacterial consortium and PPB was assessed batchwise.

2. Materials and methods

2.1 Inocula and piggery wastewater

A *Chlorella vulgaris* culture obtained from an outdoors high rate algal pond (HRAP) treating centrate was used as inoculum in the algal-bacterial photobioreactor. The PPB inoculum used was obtained from a batch enrichment in diluted PWW (17%) under continuous infrared light illumination at 50 W/m^2. Fresh PWW was collected from a nearby swine farm at Cantalejo (Spain) and stored at 4 °C. The PWW

was centrifuged for 10 min at 10000 rpm before dilution to reduce the concentration of TSS. The average composition of the 5% diluted PWW is shown in Table 1.

2.2 Batch PWW biodegradation tests

An algal-bacterial (AB) batch test was conducted in three gas-tight glass bottles of 1.1 L illuminated by LED lamps at 1380±24 μmol/m^2·s (302.2 W/m^2) for 12 hours a day. Similarly, a purple phototrophic bacteria (PPB) batch test was carried out in 1.1 L gas-tight glass bottles illuminated by IR lamps at 45±1 W/m^2 for 12 hours a day. Both light intensities were selected to simulate the natural sun radiation conditions of PAR and IR (García et al., 2017b; Hülsen et al., 2016a). The bottles were initially filled with 400 mL of 5, 10 and 15% diluted PWW and inoculated with fresh biomass at 760 mg TSS/L. The algal-bacterial inoculum, obtained from the algal-bacterial photobioreactor (PBR-AB), was composed of *Chlamydomonas* sp., *Chlorella kessieri*, *Chlorella vulgaris* and *Scenedesmus acutus*, which represented 14, 23, 43 and 20% of the algal population, respectively. Similarly, the PPB inoculum, obtained from the PPB photobioreactor (PBR-PPB), was mainly composed of bacteria from the phyla *Proteobacteria*, *Synergistetes*, *Firmicutes*, which represented 83.8, 5.3 and 3.6 % of the bacterial population, respectively.

All bottles were flushed with N_2 for 10 minutes to establish an initial environment totally deprived from O_2. The tests were incubated at 30 ºC (using thermostatic water baths) under continuous magnetic agitation (200 rpm). Liquid samples of 20 mL were periodically taken to determine the pH and concentration of total suspended solids (TSS), total organic carbon (TOC), inorganic carbon (IC) and total nitrogen (TN). In addition, gas samples from the headspace of the bottles were daily drawn using gas-tight syringes to determine by GC-TCD the gas concentration of N_2, CO_2 and O_2.

2.3 PWW biodegradation in continuous photobioreactors

The experimental set-up consisted of two 3L open photobioreactors (0.15 m deep and 0.02 m^2 of superficial area). The PBR-AB was illuminated at 1393±32 μmol/m^2·s for 12 hours a day (04h00 to 16h00) using visible LED lamps arranged in a horizontal configuration 60 cm above the surface of the PBR (Fig. 1). The PBR-PPB was illuminated at 48±4W/m^2 for 12 hours a day (04h00 to

16h00) by IR LED lamps arranged in a horizontal configuration 20 cm above the surface of the PBR (Fig. 1). The PBR-AB was jacketed and connected to a cooling water bath to maintain similar temperatures in both PBRs. The cultivation broths of PBR-AB and PBR-PPB were mixed via two water immersion pumps.

Both photobioreactors were initially filled with tap water, inoculated with fresh biomass at 275 mg TSS/L and fed with 5% diluted PWW using a 205U7CA multi-channel cassette pump (Watson-Marlow, UK) at HRTs of 10.6, 7.6 and 4.1 days in stage I, II and III, respectively (Table 1).

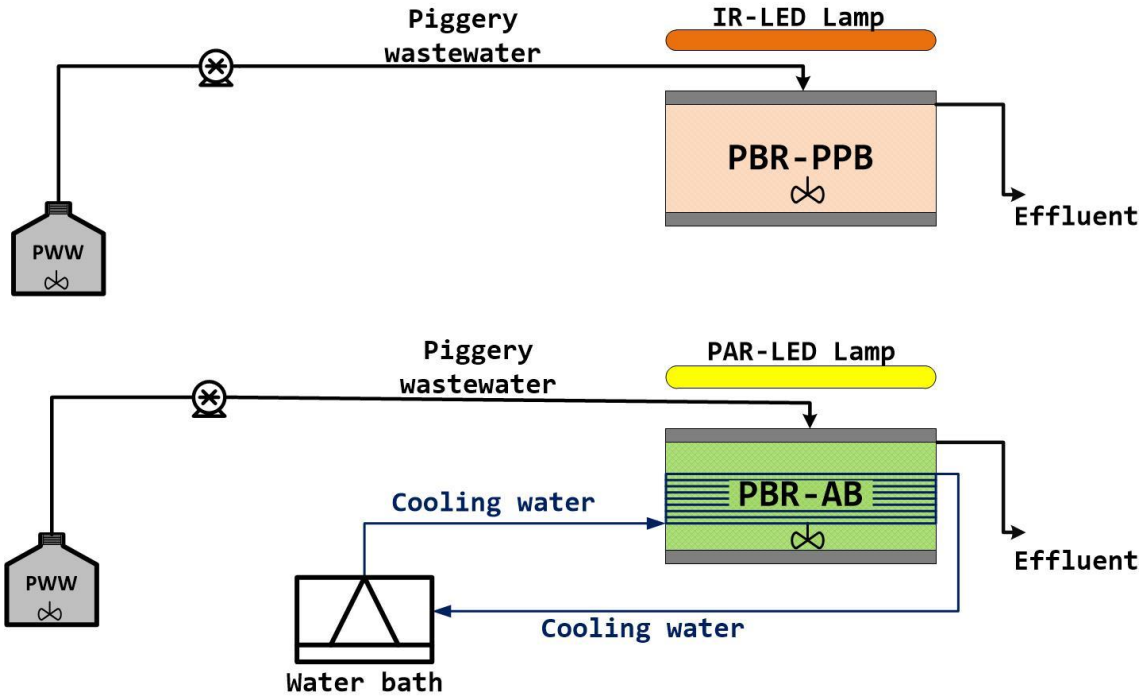


Figure 1. Schematic diagram of the algal-bacterial photobioreactor (PBR-AB) and purple photosynthetic bacteria photobioreactor (PBR-PPB) treating diluted PWW.

Liquid samples from the influent PWW and the effluents of the photobioreactors were drawn weekly to determine the concentrations of TOC, IC, TN, NH_4^+, NO_3^-, NO_2^-, TP, Zn and TSS. Likewise, the microalgae population structure in the photobioreactors was weekly assessed from biomass samples preserved with lugol acid at 5% and formaldehyde at 10%, and stored at 4 ºC prior to analysis. Cultivation broth samples from the photobioreactors were also collected under steady state conditions and immediately stored at -20 ºC to evaluate the richness and composition of the bacterial communities. Dissolved oxygen concentration (DO) and pH in the

cultivation broth of the photobioreactors were daily measured, while the influents and effluents flow rates were daily recorded to monitor water evaporation losses. Finally, the C, N and P content of the biomass was monitored in both PBRs under steady state conditions.

The removal efficiencies of C, N, P and Zn were calculated according to Eq. (1):

$$RE(\%) = \frac{(C_{inf} \times Q_{inf}) - (C_{eff} \times Q_{eff})}{C_{inf} \times Q_{inf}} \times 100 \quad (1)$$

where C_{inf} and C_{eff} represent the concentrations of TOC, IC, TN, TP and Zn in the influent PWW and PBR effluents, respectively, while Q_{inf} and Q_{eff} represent the influents and effluents flow rates, respectively. The process was considered under steady state when the TSS concentrations in the photobioreactors remained constant for at least four consecutive samplings. The results here provided correspond to the average ± standard deviation from duplicate measurements drawn weekly along one month of steady state.

2.4 Analytical procedures

A 510 pH meter (EUTECH Instrument, The Netherlands) was used to measure the pH, while a CellOX® 325 oximeter was used to measure the dissolved oxygen and temperature (WTW, Germany). PAR was measured with a LI-250A light meter (LI-COR Biosciences, Germany), while the intensity of infrared radiation was determined with a PASPort light meter (PASCO airlink®, California. USA). TOC, IC and TN concentrations were determined using a TOC-V CSH analyzer equipped with a TNM-1 module (Shimadzu, Japan). NO_2^- and NO_3^- concentrations were analyzed by HPLC-IC with a Waters 515 HPLC pump coupled with a Waters 432 ionic conductivity detector and equipped with an IC-Pak Anion HC (150 mm × 4.6 mm) Waters column (García et al., 2017a). TP, $N\text{-}NH_4^+$ and TSS concentrations were determined according to Standard Methods (APHA, 2005). The analysis of the C, N and P content in pre-dried and grinded biomass was carried out using a LECO CHNS-932 elemental analyzer. Zinc was determined using a 725-ICP Optical Emission Spectrophotometer (Agilent, USA) at 213.62 nm.

Microalgae were identified and quantified by microscopic examination

(OLYMPUS IX70, USA) according with phytoplankton manual (Sournia, 1978). Molecular analysis of the bacterial populations in PBR-AB and PBR-PPB was conducted according to García et al., (2017b). The genomic desoxyribonucleicacid (DNA) was extracted from inocula and photobioreactors effluents, respectively, by FastDNASpin Kit for Soil (MP Biomedicals, LLC, USA) according to the manufacturer's protocol. An aliquot of 300 ng DNA of each sample was provided to Fundació per al Foment de la Investigació Sanitária i Biomédica de la Comunitat Valenciana (FISABIO, Valencia, Spain) for 16S Amplicon sequencing by Illumina Miseq Platform using 926F (50-AAACTYAAAKGAATTGACGG-30) and 1392wR (50-ACGGGCGGTGWGTRC-30) primer set (Engelbrektson et al., 2010; Hülsen et al., 2016b). Raw paired reads were first trimmed by Trimmomatic to remove short (less than 190bp) and low quality reads (lower than Phred-33 of 20). Data were analyzed according to Hülsen et al., (2016b) and trimmed paired reads were then assembled by Pandaseq with default parameters. The adapter sequences were removed by FASTQ Clipper of FASTX-Toolkit. The joined high quality sequences were analysed by QIIME v1.8.0 using open-reference OTU picking strategy by uclustat 1% phylogenetic distance and assigned taxonomy by uclust against green genes database. OTUs with only one read were filtered from the OTUs table by command filter_otus_from_otu_table.py in QIIME. An *in house* script was used to find a centroid normalized OTUs table with 40,000 reads per sample. Finally, the Shannon-Wiener diversity index (*H*) was determined using the peak heights in the densitometric curves. This index, which represents both the sample richness and evenness and ranges from 1.5 to 3.5 (low and high species evenness and richness, respectively), was calculated according to Eq. (2) (MacDonald, 2003):

$$H = -\sum[P_i ln(P_i)] \quad (2)$$

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

Table 1. Operational conditions and physical/chemical characterization of the diluted PWW and cultivation broth in PBR-AB and PBR-PPB during steady state along the three operational stages.

Parameters		PWW	Stage I		Stage II		Stage III	
			PBR-AB	PBR-PPB	PBR-AB	PBR-PPB	PBR-AB	PBR-PPB
Operational days		-	84		63		77	
HRT (days)		-	≈ 10.6		≈ 7.6		≈ 4.1	
pH (units)		-	8.7±0.1	8.6±0.1	8.7±0.1	8.7±0.1	8.5±0.1	8.5±0.3
PAR (µmol/m^2.s)		-	1388±39	-	1379±33	-	1407±12	-
IR (W/m^2)		-	-	50±6	-	46±1	-	48±8
Temperature (◦C)	09h00	-	32.8±0.9	30.4±1.4	31.3±1.0	28.4±1.5	32.1±0.9	30.5±1.4
	16h00	-	31.2±1.5	30.5±1.5	30.3±1.5	29.0±1.5	30.7±1.6	30.3±1.5
Dissolved oxygen (mg/L)		-	0.04±0.02	0.05±0.02	0.03±0.02	0.02±0.01	0.03±0.02	0.03±0.02
Evaporation rates (%)		-	72±7	56±8	42±5	36±5	21±2	18±3
***TOC (mg/L)**		574±16	309±18	181±54	199±9	136±3	246±31	156±31
***IC (mg/L)**		58±4	169±17	142±3	144±10	137±7	117±13	122±15
***TN (mg/L)**		166±9	68±5	65±4	85±3	84±6	118±9	110±10
***Ammonium (mg/L)**		179±5	41±3	60±7	80±5	83±6	118±14	118±6
***Nitrate (mg/L)**		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
***Nitrite (mg/L)**		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
***TP (mg/L)**		5.65±0.41	1.62±0.61	1.35±0.51	1.62±0.43	1.75±0.27	1.22±0.27	1.22±0.57
***Zinc (mg/L)**		0.78±0.07	0.07±0.03	0.15±0.02	0.08±0.02	0.12±0.02	0.08±0.02	0.06±0.01
***TSS (mg/L)**		237±63	2640±161	873±114	1005±54	853±51	638±35	553±118
- Not applicable								
* Average value under steady stage conditions								

3. Results and Discussion

3.1 Batch PWW biodegradation tests

Overall, slightly higher TOC and TN removals were recorded in algal-bacterial tests during batch PWW treatment compared to the tests conducted with PPB. The final TOC-REs in AB tests carried out with 5, 10 and 15% diluted PWW (namely AB-5, AB-10 and AB-15, respectively) accounted for 62, 46, 64%, respectively, compared to 52, 45 and 50% in PPB tests at comparable dilutions (namely PPB-5, PPB-10 and PPB-15, respectively). AB and PPB tests experienced a rapid decrease in organic matter concentration during the first 165 hours, with no significant variation in TOC concentration until the end of the experiment (Fig. 2a). The higher organic carbon removal in AB tests can be attributed to differences in microbial population structure and the occurrence of aerobic conditions. Hence, the aerobic bacteria present in the AB consortium were likely capable of utilizing a wider spectrum of organic compounds as electron donors than PPB (Golomysova et al., 2010). The results herein obtained in AB tests were in agreement with the study conducted by de Godos et al., (2010), who recorded TOC-REs of 55, 42, 42 and 46% during the biodegradation of 8 fold diluted PWW with *E. viridis*, *S. obliquus*, *C. sorokiniana* and *Chlorella* spp, respectively, in symbiosis with activated sludge. However, Hülsen et al., (2018) reported lower total and soluble COD-REs (< 20% and < 40%, respectively) during the batch treatment of PWW by PPB.

The final removals of TN accounted for 47, 48 and 66% in AB-5, AB-10 and AB-15, respectively, and 43, 43 and 55% in PPB-5, PPB-10 and PPB-15, respectively (Fig. 2b). An active removal of nitrogen was detected during the first 165 hours of assay in both test series, which suggested the assimilatory nature of the N removal mechanism (correlated to TOC removal). The results herein obtained in AB tests were similar to those reported by de Godos et al., (2010), who recorded TN-REs ranging from 25 to 46% during the batch biodegradation of 8 fold diluted PWW. However, Hülsen et al., (2018) reported TN and NH_4^+ REs < 10% and < 40%, respectively, during the batch treatment of PWW by PPB.

On the other hand, the final biomass concentrations in AB-5, AB-10 and AB-15 accounted for 750, 1520, 2000 mg TSS/L, respectively, and 820, 1290 and

1460 mg TSS/L in PPB-5, PPB-10 and PPB-15, respectively (Fig. 2c). In this context, the higher TOC-REs and TN-REs recorded in AB tests supported the higher biomass concentrations here observed compared to PPB tests (García et al., 2017a).

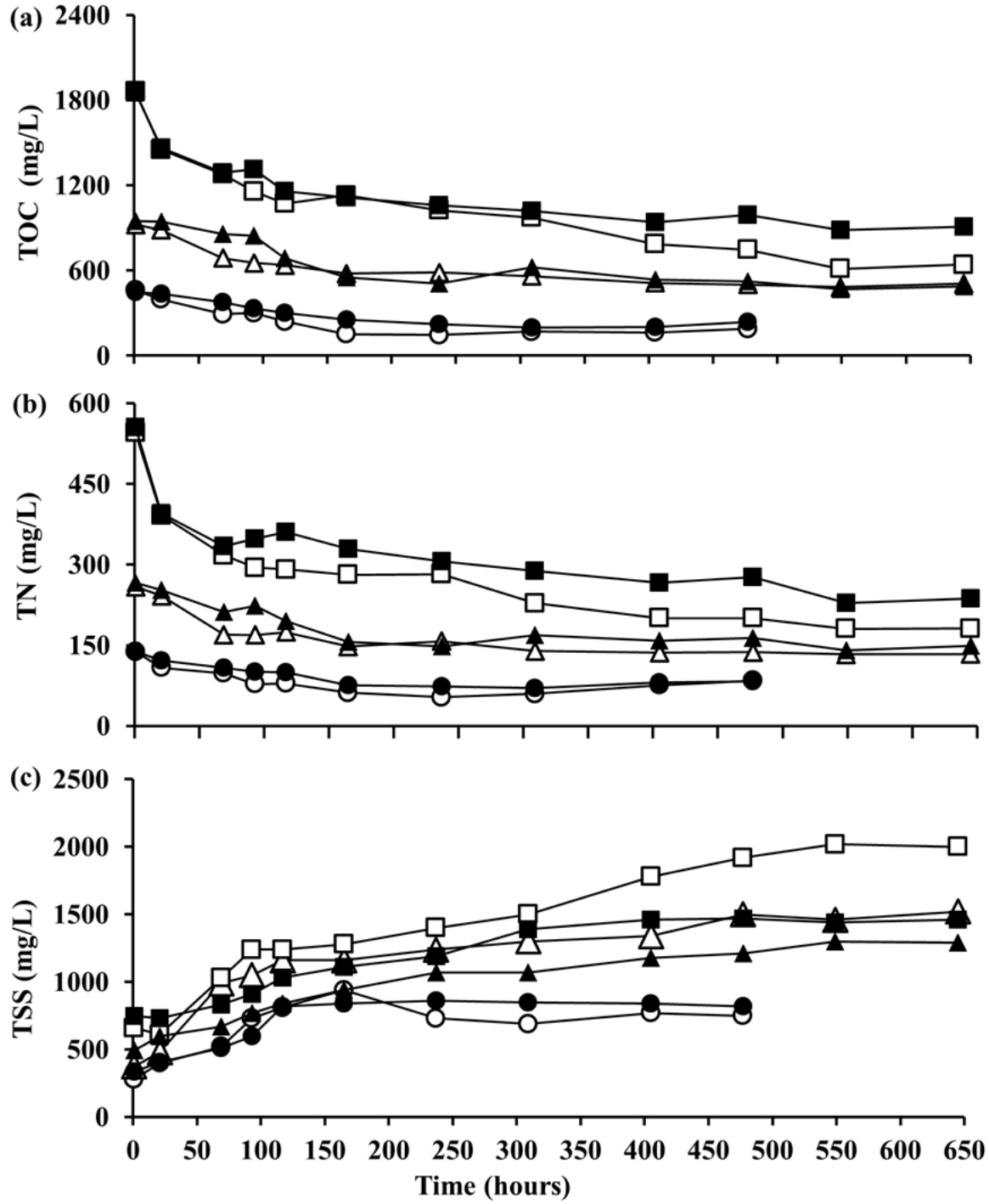


Figure 2. Time course of the concentration of TOC (**a**), TN (**b**) and TSS (**c**) in the algal-bacterial (open symbols) and purple photosynthetic bacteria **(**solid symbols) systems during the biodegradation of piggery wastewater diluted at 5% (circle), 10% (triangle) and 15% (square)**.**

3.2 PWW biodegradation in continuous photobioreactors

3.2.1 Carbon, nitrogen and phosphorous removal

A gradual deterioration in the REs of carbon and nitrogen was recorded when decreasing the HRT in both PBRs. Indeed, TOC-REs in PBR-AB averaged 84±4, 79±3 and 66±3% in SI, SII and SIII, respectively, which resulted in steady state TOC concentrations in the effluent of 309±18, 199±9 and 246±31 mg/L, respectively (Fig.3a, Table 1). The results herein obtained confirmed the consistent removals of organic matter from PWW by algal-bacterial consortia and were in agreement with García et al., (2017b), who reported TOC-REs ranging from 85 to 94% in 3 L HRAPs during the treatment of 20 and 10 folds diluted PWW at a HRT of 27 days. Likewise, Hernández et al., (2013) reported COD-REs of 62±2 % in an outdoors 5 L HRAP treating PWW at 10 days of HRT. On the other hand, the TOC-REs in PBR-PPB accounted for 87±4, 84±3 and 77±5% in SI, SII and SIII, respectively, which entailed average TOC concentrations in the effluent lower than those detected in PBR-AB: 181±54, 136±3 and 156±31 mg/L, respectively (Fig.3a, Table 1). The TOC-REs here achieved were higher than the average organic matter removals recorded by González et al., (2017) (REs ~65%) during the treatment of an anaerobic effluent in a 32 L membrane photobioreactor operated with native PPB. The high TOC-REs herein recorded in PBR-PPB could be attributed to the higher metabolic versatility of PPB, which degraded organic matter both aerobically (mediated by O_2 diffusion from the open atmosphere) and anaerobically (Hunter et al., 2009). In this context, Golomysova et al., (2010) highlighted the key role of the acetate assimilatory pathway of PPB during WWT. At this point, it must be also stressed that volatile fatty acids typically represent the main fraction of the soluble COD in PWW (González-Fernández and García-Encina, 2009). On the other hand, the steady state IC-REs in PBR-AB accounted for 5±25, -74±28 and -52±24% during SI, SII and SIII, respectively, which resulted in average IC concentrations in the effluents of 167±17, 144±10 and 177±10 mg/L, respectively (Fig.3b, Table 1). Likewise, the IC-REs in PBR-PPB averaged -15±45, -87±72 and -66±18% in SI, SII and SIII, respectively, resulting in steady state IC concentrations in the effluents of 142±3, 137±7 and 122±15 mg/L, respectively (Fig.3b, Table 1). These negative IC-REs recorded in both PBRs

evidenced the accumulation of inorganic carbon mediated by the active microbial TOC oxidation. This finding was in agreement with previous observations in HRAPs treating PWW (García et al., 2017b). Overall, the higher IC-REs during the treatment of PWW in PBR-AB were likely mediated by the higher biomass concentrations and the oxygenic nature of the photosynthesis prevailing in PBR-AB (Table 1). A carbon mass balance showed that bioassimilation was the main mechanism responsible for carbon removal in PBR-AB and PBR-PPB during SII and SIII, with C recoveries in the form of biomass ranging between 58 and 72% of the total carbon removed. Carbon removal by stripping (prior mineralization of the organic carbon to CO_2) was the main mechanism accounting for carbon removal in PBR-PPB during SI, with a contribution of 62% of the total carbon removed.

The TN-REs in PBR-AB under steady state averaged 87±2, 69±3 and 47±1% in SI, SII and SIII, respectively, which corresponded to average TN concentrations in the effluent of 68±5, 85±3, and 118±9 mg/L, respectively (Fig. 3c, Table 1). Similar results were found in PBR-PPB, where TN-REs accounted respectively for 83±2, 65±6 and 48±3%, respectively, resulting in average TN concentrations in the effluent of 65±4, 84±6, and 110±10 mg/L in SI, SII and SIII, respectively (Fig. 3c, Table 1). Steady state NH_4^+-REs of 93±1, 72±2 and 49±3% in PBR-AB and of 86±1, 68±5 and 48±4% in PBR-PPB were recorded during SI, SII, and SIII, respectively (Fig. 3d, Table 1). The TN-REs here achieved in PBR-AB were similar to those reported by García et al., (2018), who recorded TN removals of 82-85% during the treatment of 20 fold diluted PWW in indoor algal-bacterial open photobioreactors at a HRT of ≈ 27 days. However, these TN-REs were higher than the removals of 37±8% obtained by de Godos et al., (2010) during PWW treatment in a 3.5L indoor enclosed photobioreactor operated at a HRT of 4.4 days. The nitrogen mass balance conducted under steady state revealed that stripping was the main N removal mechanism in both PBRs during the three stages, with assimilation into biomass in PBR-AB accounting only for 15, 21 and 24% of the TN removed, in SI, SII and SIII, respectively. Similarly, nitrogen assimilation in PBR-PPB accounted for only 9, 19 and 29% of the TN removed in SI, SII and SIII, respectively.

Finally, TP-REs of 91±3, 84±4 and 83±3% were recorded in PBR-AB in SI, SII and SIII, respectively, which resulted in average TP concentrations in the effluent of 1.6±0.6, 1.6±0.4, and 1.2±0.3 mg/L, respectively (Fig. 3e, Table 1). On the other hand, the TP-REs in PBR-PPB accounted for 89±3, 81±1 and 82±9% in SI, SII and SIII, respectively, which entailed effluent TP concentrations of 1.3±0.5, 1.7±0.3, and 1.2±0.5 mg/L, respectively (Fig. 3e, Table 1). Interestingly, high TP-REs were recorded in both PBRs regardless of the HRT and biomass concentration. The TP-REs obtained in PBR-AB were similar to those reported by García et al., (2018) during the treatment of 15% diluted PWW in indoor algal-bacterial open photobioreactors (REs ~ from 90-92%. These values were also higher than the TP-REs of 58% reported by Myung et al., (2004) during the treatment of PWW by PPB. Phosphorus assimilation into biomass was likely the main P removal mechanism based on the moderate pH values (8.5-8-7) prevailing in both PBRs (pH = 8.5-8.7), which were not likely to support a significant phosphate precipitation (Table 1) (García et al., 2017a). Indeed, a phosphorus mass balance to PBR-AB revealed that 97, 89 and 51% of the total phosphorus removed was recovered as biomass during SI, SII and SIII, respectively. Similar P recoveries (60-81%) were estimated in PBR-PPB.

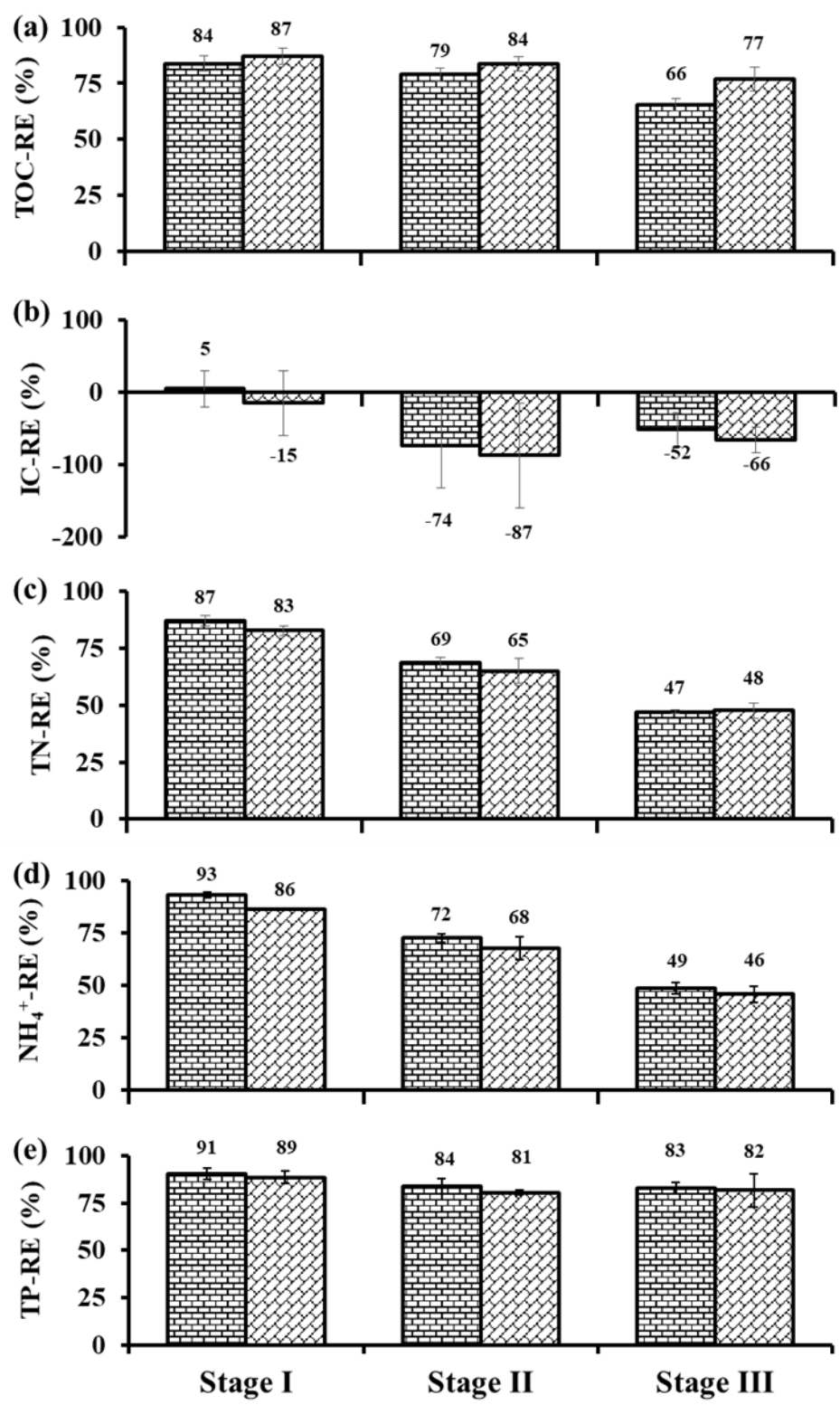


Figure 3. Steady state removal efficiencies of TOC (**a**), IC (**b**), TN (**c**), NH_4^+ (**d**) and TP (**e**) in PBR-AB (▤) and PBR-PPB (▨) in the three operational stages evaluated. Upper bold numbers indicate the steady state removal efficiencies, while vertical bars represent the standard deviation from replicate measurements during steady state operation.

3.2.2 Zinc removal

Zn-REs of 98±1, 94±2 and 91±2% were attained in PBR-AB in SI, SII and SIII, respectively, which mediated very low concentrations of Zn in the effluent under steady state conditions (0.07 ± 0.03, 0.08±0.02 and 0.08 ± 0.02 mg/L, respectively) (Table 1). On the other hand, the Zn -REs in PBR-PPB accounted for 93±1, 90±2 and 92±2% in SI, SII and SIII, respectively, which resulted in Zn effluent concentrations of 0.15±0.02, 0.12±0.02 and 0.06±0.01 mg/L, respectively (Fig. 3e, Table 1). The moderate pH prevailing in both PBRs (8.5-8-7) suggest that biosorption was likely the main mechanism governing Zn removal, although Zn-REs were not correlated with biomass concentrations (Javanbakht et al., 2014; Kaplan et al., 1987). The Zn-REs herein achieved were higher than those reported by García et al. (2017b) during PWW treatment in 3 L indoors HRAPs operated at a HRT of ≈27 days (71-83%).

3.2.3 Concentration, productivity and composition of biomass

Biomass concentration in PBR-AB increased during SI from ≈237 mg TSS/L up to steady state concentrations of 2640±161 mg TSS/L by days 63-84. A rapid decrease of biomass concentration to 1005±54 mg TSS/L in SII and to 683±35 mg TSS/L in SIII occurred as a result of the stepwise decrease in HRT in PBR-AB (Fig. 4a, Table 1). On the other hand, the biomass concentration in PBR-PPB experienced a gradual increase during the first 28 days of operation and stabilized at 873±114 mg TSS/L. Despite the decrease in HRT from 10.6 to 7.6 days did not result in a significant variation in TSS concentration in PBR-PPB (853±51 mg TSS/L in SII), a gradual decrease to 553±118 mg TSS/L occurred during SIII mediated by the decrease in HRT to 4.1 days (Fig.4a). The higher biomass concentrations recorded in PBR-AB compared to PBR-PPB, which were more evident during SI, were likely due to the active photosynthetic CO_2 assimilation by microalgae. These differences in biomass concentrations between both PBRs could be also explained by the slightly higher evaporation rates recorded in PBR-AB induced by its slightly higher temperatures. Hence, water evaporation (estimated as the ratio between the flow rate of water evaporation and the influent flow rate) in SI, SII and SIII accounted for 72, 42 and 21% in PBR-AB, and 56, 36 and 18% in PBR-PPB, respectively.

The lowest areal biomass productivities were recorded in SI in both PBRs, accounting for 10.4 and 5.4 g/m^2·d in PBR-AB and PBR-PPB, respectively. The decrease in HRT resulted in increased biomass productivities up to 11.5 and 10.8 g/m^2·d in stage II in PBR-AB and PBR-PPB, respectively. Finally, process operation at a HRT of 4.1 days was characterized by the highest biomass productivities: 18.4 g/m^2·d in PBR-AB and 16.6 g/m^2·d in PBR-PPB.

The C, N and P content of the algal-bacterial biomass averaged 48 ± 4, 7.5 ± 1.4 and 0.62 ± 0.15%, and 52 ± 1, 8.4 ± 0.5 and 0.69± 0.05% in the PPB biomass, with no clear correlation with the HRT (Fig. 4b). The algal-bacterial biomass composition was similar to the values reported by Cabanelas et al., (2013), who determined a C, N and P content in the harvested biomass of ≈ 44, 7.5 and 0.5%, respectively, in a photobioreactor inoculated with *C. vulgaris* and supplemented with CO_2 during the treatment of settled domestic wastewater.

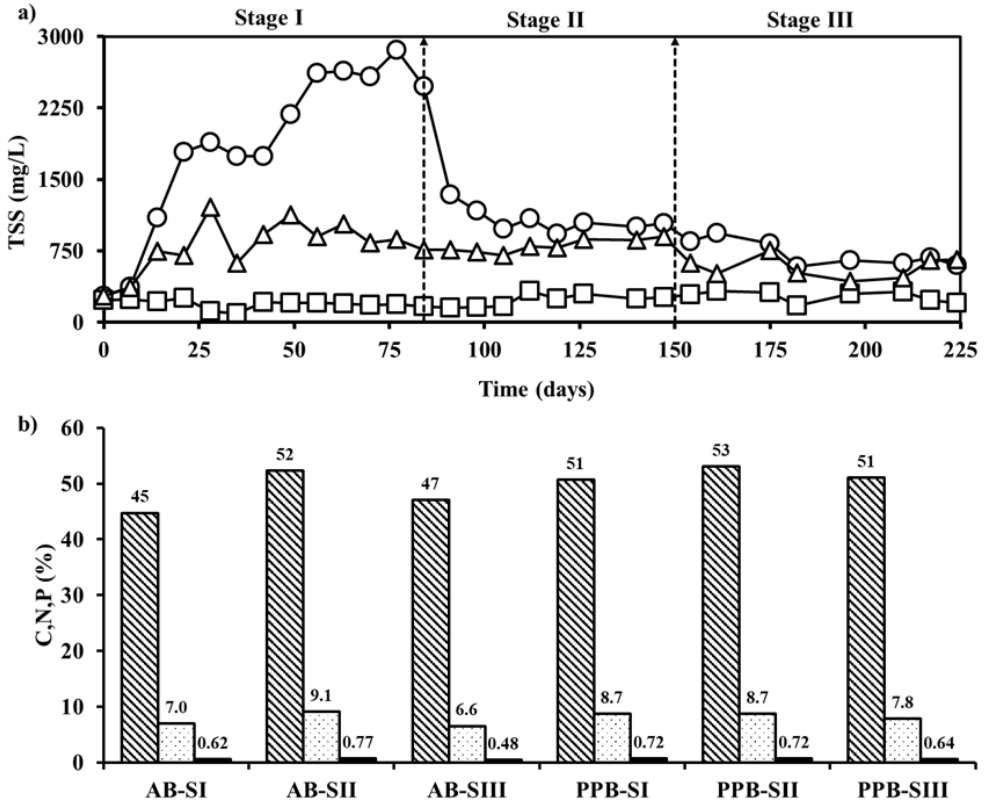


Figure 4. a) Time course of TSS concentration in PWW (□), PBR-AB (○) and PBR-PPB (Δ) during the entire experiment. **b)** C (▨), N (▭) and P (■) content in the biomass present in the photobioreactors under steady state**.**

3.2.4 Microalgae population structure

C. vulgaris represented the dominant species in PBR-AB from day 1 to day 84 in SI, with a maximum concentration of $1.6 \cdot 10^{10}$ cells/L by day 35 (corresponding to 90 % of the total cell number) (Fig. 5a). Interestingly, a severe decrease in the total number of microalgae cells was observed from day 35 to day 56, which remained stable at $1.8 \pm 0.2 \cdot 10^{9}$cells/L by the end of SI. *Chlorella kessieri* was always detected from day 42 to day 84, while *Scenedesmus acutus* and *Tetradesmus obliquus* were detected for 10 and 7 weeks during SI, respectively. On the other hand, no microalgae was detected in PBR-PPB during SI (Fig. 5b). During SII, *C. vulgaris* and *C. kessieri* were present in PBR-AB throughout the experimental period from day 91 to day147. *C. vulgaris* achieved a maximum concentration of $0.24 \cdot 10^{9}$ cells/L by day 105 (corresponding to 55 % of the total cell number). However, the maximum cell concentration was recorded by day 119, where $0.51 \cdot 10^{9}$cells/L and eight microalgae species were detected. S*c. acutus* and *Tet. obliquus (acutudesmus)* were not detected from days 98 and 126 onwards, respectively. On the other hand, *C. vulgaris* and *Chlorococcum sp.* were detected 6 and 2 times during SII in PBR-PPB, respectively. However, the maximum microalgae cell concentration in PBR-PPB was only $0.01 \cdot 10^{9}$ cells/L, which was recorded by day 147 as a result of the occurrence of four microalgae species. Finally, *C. vulgaris* and *C. kessieri* were always present in SIII from day 154 to day 224 in PBR-AB, while *Chlorella minutissima* was identified from day 175 onwards. However, the maximum cell concentration of microalgae in PBR-AB during SIII was only $0.27 \cdot 10^{9}$cells/L (day 217). Finally, six microalgae species were detected in PBR-PPB during SIII. *Aphanothece saxicola* was detected by day 161 and day 217, while *C. vulgaris*, *C. kessieri* and *C. minutissima* were detected during SIII up to days 154, 161 and 182, respectively. The N_2 fixing cyanobacteria, *Cyanobium* spp. and *Pseudanabaena rosea* were dominant by the end of SIII, when the maximum microalgae concentration ($0.19 \cdot 10^{9}$ cells/L by day 217) was achieved (Richmond, 2004).

C. vulgaris, the microalga species inoculated in PBR-AB, was detected in all stages from day 7 to day 224, along with other *Chlorella* species. The high tolerance of microalgae from the genus *Chlorella*, which ranked 5th in the

ranking of pollution tolerant microalgae species established by Palmer (1969), supported the dominance of this microalgal species in PBR-AB regardless of the HRT. In addition, the monitoring of the microalgae population structure clearly showed that the decrease in HRT induced a gradual wash-out of microalgae, which mediated a significant decrease in the number of cells from 1.8 cells/L·10^9 (day 84) to 0.17 cells/L·10^9 (day 244) under steady state in PBR-AB. This study also revealed that inoculation of the PBR-AB with a specific photosynthetic microorganism does not guarantee its long-term dominance during PWW treatment (Serejo et al., 2015). Interestingly, the stepwise decrease in HRT in PBR-PPB induced the occurrence of microalgae from day 98 onwards. Finally, it should be stressed that no direct correlation between the structure of microalgae population in the PBRs and the structure of bacterial population was found (Fig. 4, 5).

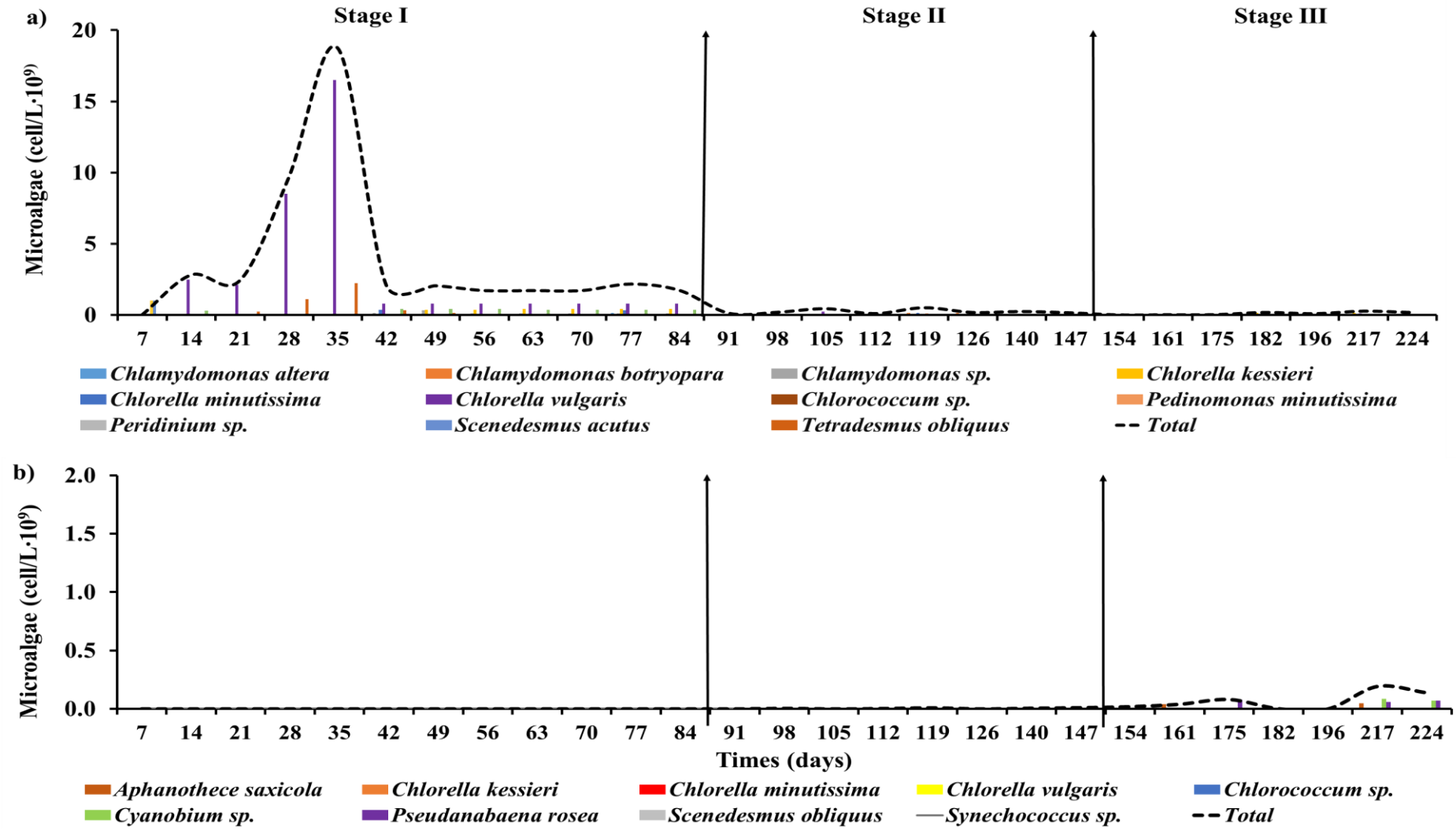


Figure 5 Time course of the microalgae population structure in PBR-AB (**a**) and PBR-PPB (**b**) during the three operational stages.

3.2.5 Bacteria population structure

The bacterial analysis of the microbial communities present in PBR-AB revealed the occurrence of the following phyla along the three operational stages: *Actinobacteria, Chloroflexi, Cyanobacteria, Epsilonbacteraeota, Firmicutes, Patescibacteria and Proteobacteria* among others phyla. *Proteobacteria and Cyanobacteria* represented the main phyla present in the inoculum of PBR-AB, with shares of 67.1 and 26.9, respectively. All phyla were detected in SI and SII in PBR-AB under steady state, although the phylum *Actinobacteria* was not present in SIII. The decrease in HRT induced a severe swift in the structure of the bacterial community, represented by *Firmicutes* (43.8%), *Epsilonbacteraeota* (10.7%), *Chloroflexi* (9.3%) *Proteobacteria* (10.9%) and *Cyanobacteria* (13.2%) at the end of the experiment (Table 2). On the other hand, the bacterial analysis in PBR-PPB revealed the occurrence of the phyla *Acidobacteria, Chloroflexi, Epsilonbacteraeota, Firmicutes, Patescibacteria, Proteobacteria and Synergistetes* among others. *Proteobacteria* and *Synergistetes* accounted for 83.8 and 5.3% of the bacteria in the inoculum of PBR-PPB. *Epsilonbacteraeota, Firmicutes and Proteobacteria* were dominant along the three stages, while *Patescibacteria, w*hich was present in SI and SII, was not detected in SIII. The decrease in HRT in PBR-PPB mediated the dominance of *Firmicutes* and *Epsilonbacteraeota* (46.7 % and 19.8 % of abundance) and decreased the contribution of *Proteobacteria* to 19.8% in SIII (Table 2). Overall, the HRT seems to play a key role on the bacterial population structure in both PBR-AB and PBR-PPB during PWW treatment. *Firmicutes* is one of two dominant phyla in the large intestine of human and pig (Ban-Tokuda et al., 2017). *Firmicutes* can degrade volatile fatty acids, which typically account for 80% of the TOC in the soluble fraction of PWW (Ferrero et al., 2012).

Table 2. Taxonomic report of the bacteria present in PBR-AB and PBR-PPB.

Phyllum	PBR-AB (%)				PBR-PPB (%)			
	Inoc.	SI	SII	SIII	Inoc.	SI	SII	SIII
Acidobacteria						0.5	2.5	1.5
Actinobacteria		1.3	3.4					
Chloroflexi		13.2	11.3	9.3			3.7	5.4
Cyanobacteria	26.9	25.7	3.1	13.2				
Epsilonbacteraeota		18.8	8.2	10.7		46.6	19.9	19.8
Firmicutes	1.0	4.7	38.2	43.8	3.6	5.3	29.3	46.7
Patescibacteria		5.5	0.6	1.0	0.0	4.5	6.5	
Proteobacteria	67.1	19.1	21.6	10.9	83.8	30.5	24.6	12.1
Synergistetes					5.3			
Other	5.0	11.8	13.5	11.1	7.3	12.6	13.5	14.4
Total nº of Cells	34903	18964	13683	28884	44503	29644	19355	14765

Proteobacteria was the main phylum with 67.1 and 83.8 % of the species present in the inocula of PBR-AB and PBR-PPB, respectively (Fig. 6). Interestingly, *Alphaproteobacteria* was the only class found in both photobioreactors. *Blastomonas,* which are aerobic and catalase/oxidase-positive, was the dominant genus in the inoculum of PBR-AB with 49.7% of the total number of bacteria (Castro et al., 2017). However, *Blastomonas* was not found in PBR-AB during SI, SII and SIII. *Rhodoplanes* was detected in PBR-AB during SII (18.7% of the total number of bacteria) and SIII (1.5%), while *Rhodobacter* was only present in SIII (6.5%), despite both genera belong to PPB (Hunter et al., 2009)(Hiraishi and Ueda, 1994)(Fig. 6a). On the other hand, *Rhodopseudomonas* was the dominant genus in the inoculum of PBR-PPB with 81.7% of the total number of bacteria, but disappeared from SII onwards (Fig. 6b). *Rhodopseudomonas* are purple non-sulfur phototropic bacteria (Hiraishi and Ueda, 1994) that can metabolize organic substrates (Cheah et al., 2016). The phototrophic *Rhodoplanes* accounted for 9.2, 11.4 and 7.5% of the total number of bacteria in SI, SII and SIII, respectively (Hunter et al., 2009) (Hiraishi and Ueda, 1994). In this context, a wash-out of *Rhodopseudomonas* followed by the dominance of *Rhodoplanes* was also observed by Chitapornpan et al., (2013) in a membrane PPB-based photobioreactor during the treatment of food processing wastewater.

Finally, the Shannon-Wiener diversity indexes calculated in both photobioreactors indicated an increase in diversity compared to the inocula, which

remained similar during process operation. Thus, the values of *H* in the inoculum, SI, SII and SIII were, respectively, 0.74, 1.89, 1.64 and 1.69 in PBR-AB, and 0.53, 1.32, 1.68 and 1.48 in PBR-PPB. This low-bacterial diversity in both photobioreactors was likely due to the high toxicity of the PWW treated and the low HRT used in this study.

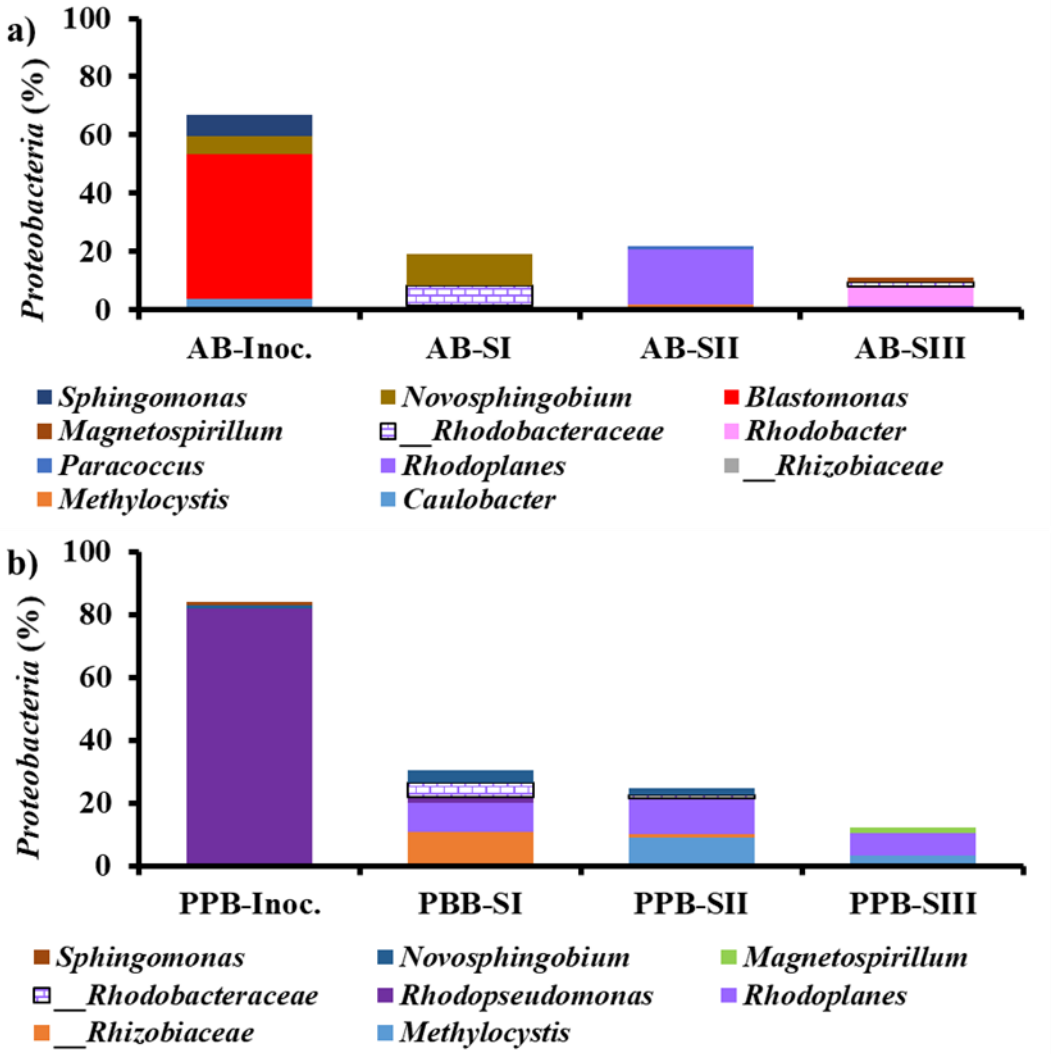


Figure 6. Relative abundance (%) of genera belonging to the phylum *Proteobacteria* in the inocula and cultivation broth of PBR-AB (**a**) and PBR-PPB (**b**) along the three operational stages. The abundance was calculated based on the total number of bacteria.

4. Conclusions

This work constitutes, to the best of our knowledge, the first comparative evaluation of the potential of microalgae and PPB during continuous PWW treatment in open photobioreactors. This research revealed a similar treatment performance of both photosynthetic microorganisms in terms of carbon,

nutrients and zinc removal. The PBR-PPB exhibited a slightly better capacity to remove organic matter, which was not observed during batch PWW treatment. Interestingly, a superior carbon and nutrient recovery was recorded in PBR-AB. The stepwise decrease in HRT, rather than the type of illumination used, caused significant changes in the structure of microalgae and bacterial population.

Acknowledgments

This research was supported by the Spanish Ministry of Science, Innovation and Universities, the FEDER EU program (CTQ2017-84006-C3-1-R), and the Regional Government of Castilla y León (UIC 71). The financial support from the program EURICA (Erasmus Mundus Action 2, Strand 1, Lot 15, Grant Agreement number 2013-2587) and Universidad Nacional Autónoma de Nicaragua (UNAN-Managua) is also gratefully acknowledged.

References

1. APHA, 2005. Standards Methods for the Examination of Water and Wastewater, 21 st. ed. American Public Health Association, American Water Works Association, Water Enviroment Federation, Washington,D.C.

2. Ban-Tokuda, T., Maekawa, S., Miwa, T., Ohkawara, S., Matsui, H., 2017. Changes in faecal bacteria during fattening in finishing swine. Anaerobe 47, 188–193. doi:10.1016/j.anaerobe.2017.06.006

3. Bastos, R.G., Queiroz, Zepka, L.Q., Volpato, G., García, Jacob-Lopes, 2009. COD Removal of Parboilized Rice Wastewater By Cyanobacteria Aphanothece Microscopica Nägeli. BioEng, Campinas 3, 245–250.

4. Bertling, K., Hurse, T.J., Kappler, U., Rakic, A.D., Sciences, M., 2006. Lasers — An Effective Artificial Source of Radiation for the Cultivation of Anoxygenic Photosynthetic Bacteria. Biotechnology and Bioengineering 94, 337–345. doi:10.1002/bit

5. Cabanelas, I.T.D., Ruiz, J., Arbib, Z., Chinalia, F.A., Garrido-Pérez, C., Rogalla, F., Nascimento, I.A., Perales, J.A., 2013. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresource Technology 131, 429–436. doi:10.1016/j.biortech.2012.12.152

6. Castro, D.J., Llamas, I., Béjar, V., Martínez-Checa, F., 2017. Blastomonas quesadae sp. Nov., isolated from a saline soil by dilution-to-extinction cultivation. International Journal of Systematic and Evolutionary Microbiology 67, 2001–2007. doi:10.1099/ijsem.0.001902

7. Cheah, W.Y., Ling, T.C., Show, P.L., Juan, J.C., Chang, J.S., Lee, D.J., 2016. Cultivation in wastewaters for energy: A microalgae platform. Applied Energy 179, 609–625. doi:10.1016/j.apenergy.2016.07.015

8. Chitapornpan, S., Chiemchaisri, C., Chiemchaisri, W., Honda, R., Yamamoto, K., 2013. Organic carbon recovery and photosynthetic bacteria population in an anaerobic membrane photo-bioreactor treating food processing wastewater. Bioresource

Technology 141, 65–74. doi:10.1016/j.biortech.2013.02.048

9. Craggs, R., Sutherland, D., Campbell, H., 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. Journal of Applied Phycology 24, 329–337. doi:10.1007/s10811-012-9810-8

10. Dassey, A.J., Theegala, C.S., 2013. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresource Technology 128, 241–245. doi:10.1016/j.biortech.2012.10.061

11. de Godos, I., Arbib, Z., Lara, E., Rogalla, F., 2016. Evaluation of High Rate Algae Ponds for treatment of anaerobically digested wastewater: Effect of CO2addition and modification of dilution rate. Bioresource Technology 220, 253–261. doi:10.1016/j.biortech.2016.08.056

12. de Godos, I., Vargas, V.A., Blanco, S., González, M.C.G., Soto, R., García-Encina, P.A., Becares, E., Muñoz, R., 2010. A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. Bioresource Technology 101, 5150–5158. doi:10.1016/j.biortech.2010.02.010

13. de Lima, L.K.F., Ponsano, E.H.G., Pinto, M.F., 2011. Cultivation of Rubrivivax gelatinosus in fish industry effluent for depollution and biomass production. World Journal of Microbiology and Biotechnology 27, 2553–2558. doi:10.1007/s11274-011-0725-3

14. Engelbrektson, A., Kunin, V., Wrighton, K.C., Zvenigorodsky, N., Chen, F., Ochman, H., Hugenholtz, P., 2010. Experimental factors affecting PCR-based estimates of microbial species richness and evenness. The ISME Journal 4, 642–647. doi:10.1038/ismej.2009.153

15. Ferrero, E.M., de Godos, I., Rodríguez, E.M., García-Encina, P.A., Muñoz, R., Bécares, E., 2012. Molecular characterization of bacterial communities in algal-bacterial photobioreactors treating piggery wastewaters. Ecological Engineering 40, 121–130. doi:10.1016/j.ecoleng.2011.10.001

16. Franchino, M., Tigini, V., Varese, G.C., Mussat Sartor, R., Bona, F., 2016. Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. Science of The Total Environment 569, 40–45. doi:10.1016/j.scitotenv.2016.06.100

17. García, D., Alcántara, C., Blanco, S., Pérez, R., Bolado, S., Muñoz, R., 2017a. Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading. Chemical Engineering Journal 313, 424–434. doi:10.1016/j.cej.2016.12.054

18. García, D., Posadas, E., Blanco, S., Acién, G., García-Encina, P., Bolado, S., Muñoz, R., 2018. Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors. Bioresource Technology 248, 120–126. doi:10.1016/j.biortech.2017.06.079

19. García, D., Posadas, E., Grajeda, C., Blanco, S., Martínez-Páramo, S., Acién, G., García-Encina, P., Bolado, S., Muñoz, R., 2017b. Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions. Bioresource Technology 245, 483–490. doi:10.1016/j.biortech.2017.08.135

20. Golomysova, A., Gomelsky, M., Ivanov, P.S., 2010. Flux balance analysis of

photoheterotrophic growth of purple nonsulfur bacteria relevant to biohydrogen production. International Journal of Hydrogen Energy 35, 12751–12760. doi:10.1016/j.ijhydene.2010.08.133

21. González-Fernández, C., García-Encina, P.A., 2009. Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry. Biomass and Bioenergy 33, 1065–1069. doi:10.1016/j.biombioe.2009.03.008

22. González, E., Díaz, O., Ruigómez, I., de Vera, C.R., Rodríguez-Gómez, L.E., Rodríguez-Sevilla, J., Vera, L., 2017. Photosynthetic bacteria-based membrane bioreactor as post-treatment of an anaerobic membrane bioreactor effluent. Bioresource Technology 239, 528–532. doi:10.1016/j.biortech.2017.05.042

23. Hernández, D., Riaño, B., Coca, M., García-González, M.C., 2013. Treatment of agro-industrial wastewater using microalgae-bacteria consortium combined with anaerobic digestion of the produced biomass. Bioresource Technology 135, 598–603. doi:10.1016/j.biortech.2012.09.029

24. Hiraishi, A., Ueda, Y., 1994. Rhodoplanes gen. nov., a New Genus of Phototrophic Bacteria Including Rhodopseudomonas rosea as Rhodoplanes roseus comb. nov. and Rhodoplanes elegans sp. nov. International Journal of Systematic Bacteriology 44, 665–673. doi:10.1099/00207713-44-4-665

25. Hülsen, T., Barry, E.M., Lu, Y., Puyol, D., Batstone, D.J., 2016a. Low temperature treatment of domestic wastewater by purple phototrophic bacteria: Performance, activity, and community. Water Research 100, 537–545. doi:10.1016/j.watres.2016.05.054

26. Hülsen, T., Barry, E.M., Lu, Y., Puyol, D., Keller, J., Batstone, D.J., 2016b. Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor. Water Research 100, 486–495. doi:10.1016/j.watres.2016.04.061

27. Hülsen, T., Batstone, D.J., Keller, J., 2014. Phototrophic bacteria for nutrient recovery from domestic wastewater. Water Research 50, 18–26. doi:10.1016/j.watres.2013.10.051

28. Hülsen, T., Hsieh, K., Lu, Y., Tait, S., Batstone, D.J., 2018. Simultaneous treatment and single cell protein production from agri-industrial wastewaters using purple phototrophic bacteria or microalgae – A comparison. Bioresource Technology 254, 214–223. doi:10.1016/j.biortech.2018.01.032

29. Hunter, C.N., Daldal, F., Thurnauer, M.C., Beatty, J.T., 2009. The Purple Phototrophic Bacteria. doi:10.1007/978-1-4020-8815-5

30. Javanbakht, V., Alavi, S.A., Zilouei, H., 2014. Mechanisms of heavy metal removal using microorganisms as biosorbent. Water Science and Technology 69, 1775–1787. doi:10.2166/wst.2013.718

31. Kantachote, D., Torpee, S., Umsakul, K., 2005. The potential use of anoxygenic phototrophic bacteria for treating latex rubber sheet wastewater. Electronic Journal of Biotechnology 8, 314–323. doi:10.2225/vol8-issue3-fulltext-8

32. Kaplan, D., Cristiaen, D., Shoshana, A., 1987. Chelating Properties of Extracellular Polysaccharides from Chlorella spp. Applied and Environmental Microbiology. 53, 2953–2956.

33. Madukasi, E.I., Dai, X., He, C., Zhou, J., 2010. Potentials of phototrophic bacteria in treating pharmaceutical wastewater. Int. J Environ. Sci. Tech 7, 165–174.

34. Mateo-sagasta, J., Burke, J., 2012. Agriculture and water quality interactions: a

global overview. SOLAW Background Thematic Report - TR08.

35. Myung, K.K., Choi, K.M., Yin, C.R., Lee, K.Y., Im, W.T., Ju, H.L., Lee, S.T., 2004. Odorous swine wastewater treatment by purple non-sulfur bacteria, Rhodopseudomonas palustris, isolated from eutrophicated ponds. Biotechnology Letters 26, 819–822. doi:10.1023/B:BILE.0000025884.50198.67

36. Oswald, W.J., Gotaas, H.B., Golueke, C.G., Kellen, W.R., Gloyna, E.F., Hermann, E.R., 1957. Algae in Waste Treatment [with Discussion]. Water Environment Federation 29, 437–457. doi:10.2307/25033322

37. Palmer, C.M., 1969. A composite rating of algae tolerating organic pollution. J. Phicol 5, 78–82.

38. Posadas, E., Marín, D., Blanco, S., Lebrero, R., Muñoz, R., 2017. Simultaneous biogas upgrading and centrate treatment in an outdoors pilot scale high rate algal pond. Bioresource Technology 232, 133–141. doi:10.1016/j.biortech.2017.01.071

39. Richmond, A., 2004. Handbook of microalgal culture: biotechnology and applied phycology/edited by Amos Richmond. doi:10.1002/9780470995280

40. Rittmann, B.E., McCarty, P.L., 2012. Environmental Biotechnology:Principles and Applications, 1 st. ed. Tata McGraw-Hill, New Delhi.

41. Serejo, M.L., Posadas, E., Boncz, M.A., Blanco, S., García-Encina, P., Muñoz, R., 2015. Influence of biogas flow rate on biomass composition during the optimization of biogas upgrading in microalgal-bacterial processes. Environmental Science and Technology 49, 3228–3236. doi:10.1021/es5056116

42. Sournia, A., 1978. Phytoplankton manual. UNESCO, Paris.

43. statista, 2018. Global pork production in 2017, by country [WWW Document]. URL https://www.statista.com (accessed 5.10.18).

44. Tigini, V., Franchino, M., Bona, F., Varese, G.C., 2016. Is digestate safe? A study on its ecotoxicity and environmental risk on a pig manure. The Science of the total environment 551–552, 127–132. doi:10.1016/j.scitotenv.2016.02.004

45. Zhang, D., Yang, H., Zhang, W., Huang, Z., 2003. Rhodocista pekingensis sp . nov ., a cyst-forming phototrophic bacterium from a municipal wastewater treatment plant. International Journal of Systematic and Evolutionary Microbiology 53, 1111–1114. doi:10.1099/ijs.0.02500-0

SUPPLEMENTARY MATERIAL

A systematic comparison of the potential of a microalgal-bacterial consortium and purple phototrophic bacteria for the continuous treatment of piggery wastewater

Dimas García[1,2,3], Ignacio de Godos[1,2,5], Christian Domínguez[1], Sara Turiel[4], Silvia Bolado[1,2], Raúl Muñoz[1,2]*

[1]Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

[2]Institute of Sustainable Processes, Dr. Mergelina, s/n, 47011, Valladolid, Spain

[3]Centro para la Investigación de los Recursos Acuáticos de Nicaragua, CIRA/UNAN-Managua, Apdo. Postal 4598, Nicaragua

[4]The Institute of the Environment, La Serna 58 – 24007, León, Spain

[5]School of Forestry, Agronomic and Bioenergy Industry Engineering (EIFAB), Valladolid University, Campus Duques de Soria, Soria, Spain.

*corresponding author: mutora@iq.uva.es

CONTENT

- Table S3;
- Table S4;
- Table S5;
- Table S6;
- Table S7
-

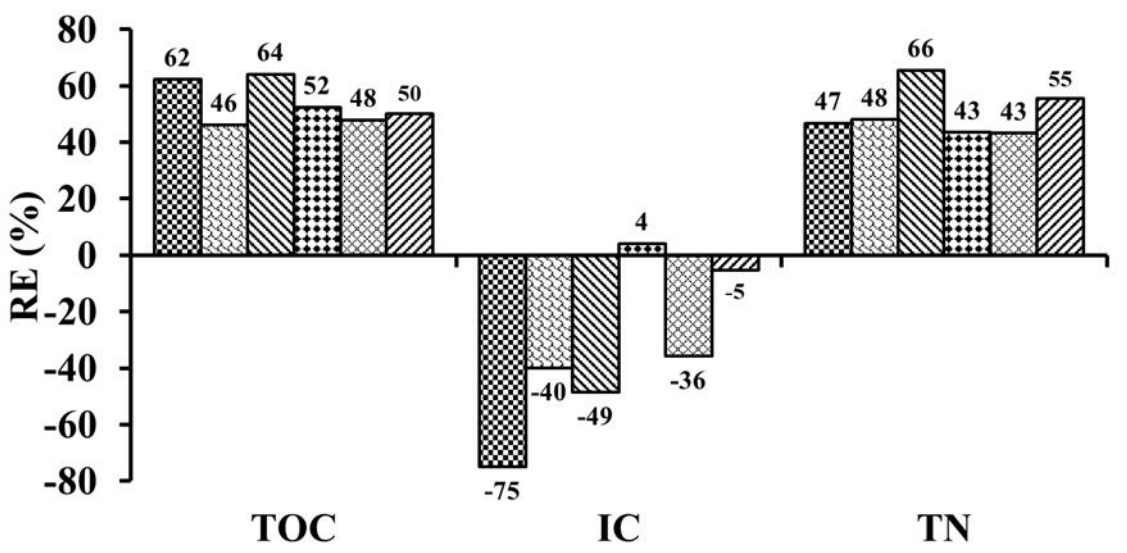


Figure S1. Average removal efficiencies of TOC, IC and TN under steady state for AB-5 (), AB-10 (), AB-15 (), PPB-5 (), PPB-10 () and PPB-15 (). Bold numbers indicate the steady state removal efficiencies.

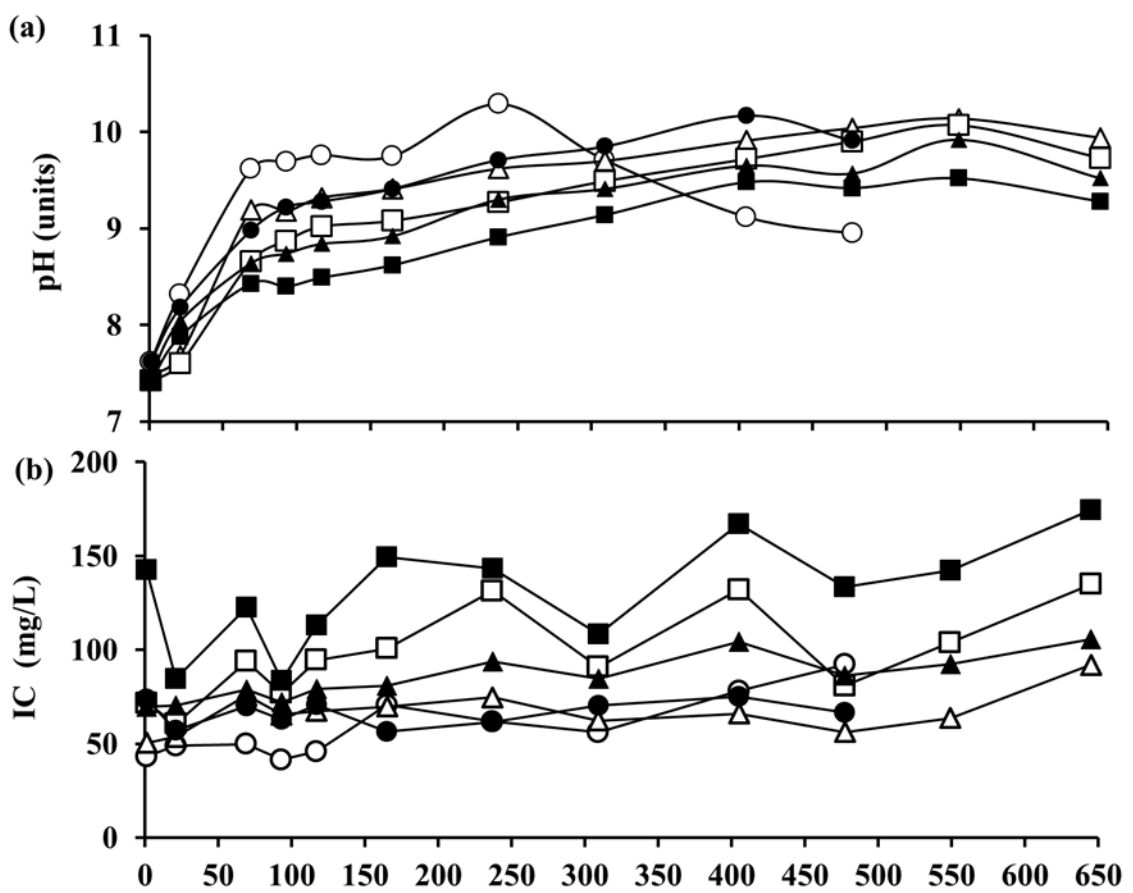


Figure S2. Time course of pH (**a**) and IC (**b**) in AB (open symbols) and PPB **(**solid symbols) tests during the batch treatment of PWW diluted at 5% (circle), 10% (triangle) and 15% (square).

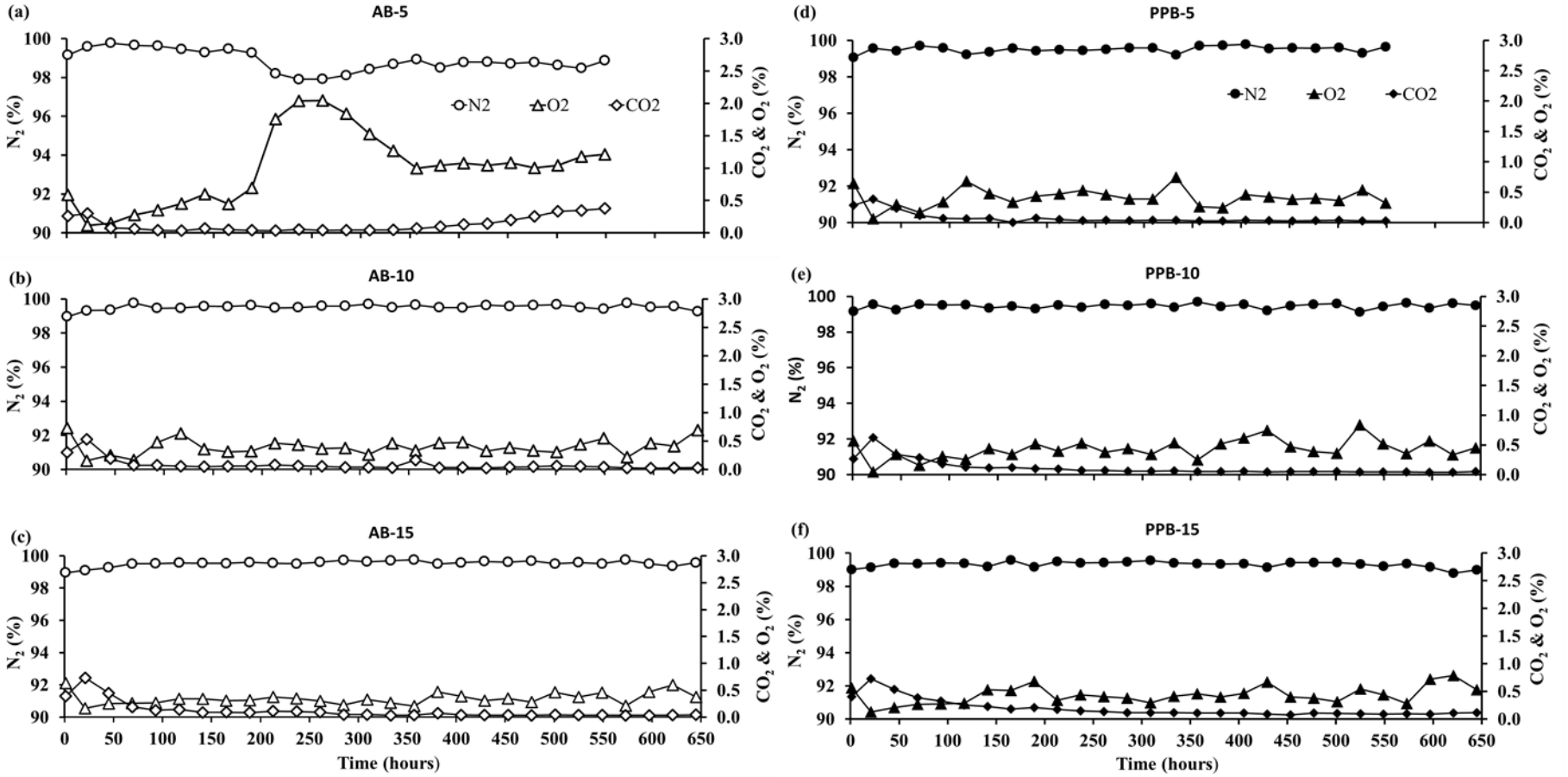


Figure S3. Time course of the headspace concentrations of N_2, CO_2 and O_2 in AB (**a**, **b** and **c**) and PPB (**d**, **e** and **f**) tests during the batch treatment of PWW diluted at 5% (AB-5 and PB-5), 10% (AB-10 and PB-10) and 15% (AB-15 and PB-15).

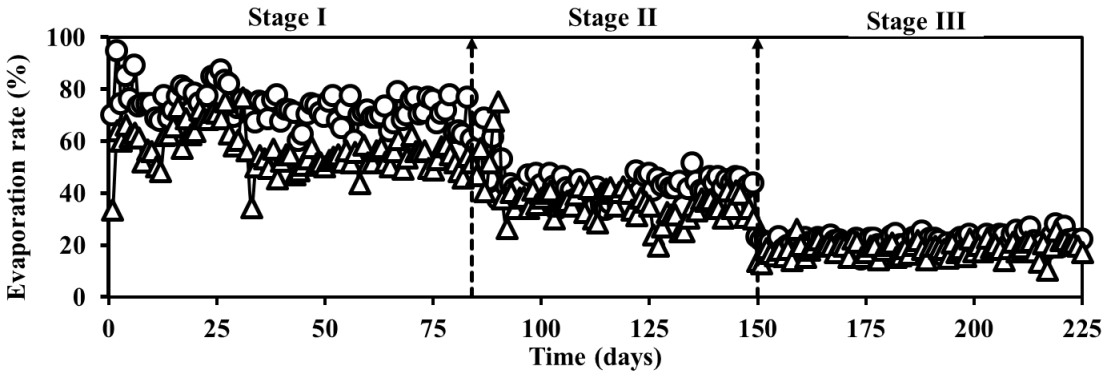


Figure S4. Time course of the evaporation rates in PBR-AB (○) and PBR-PPB (Δ).

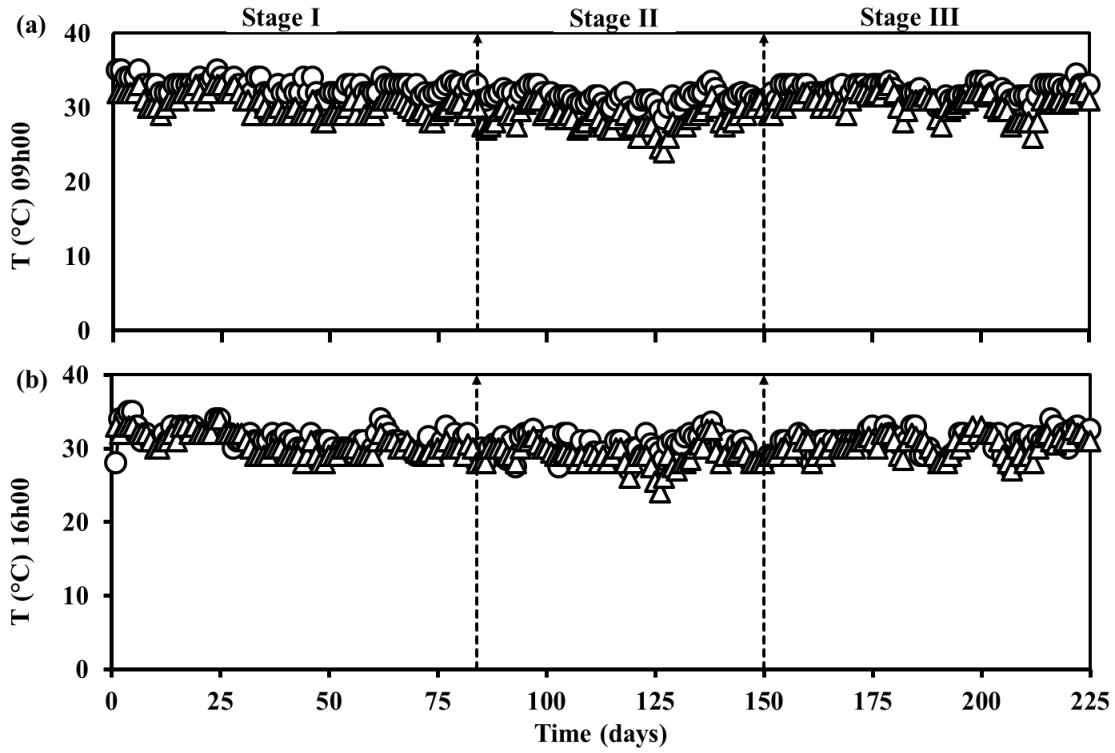


Figure S5. Time course of the temperature at 09h00 (**a**) and 16h00 (**b**) in PBR-AB (○) and PBR-PBB (Δ).

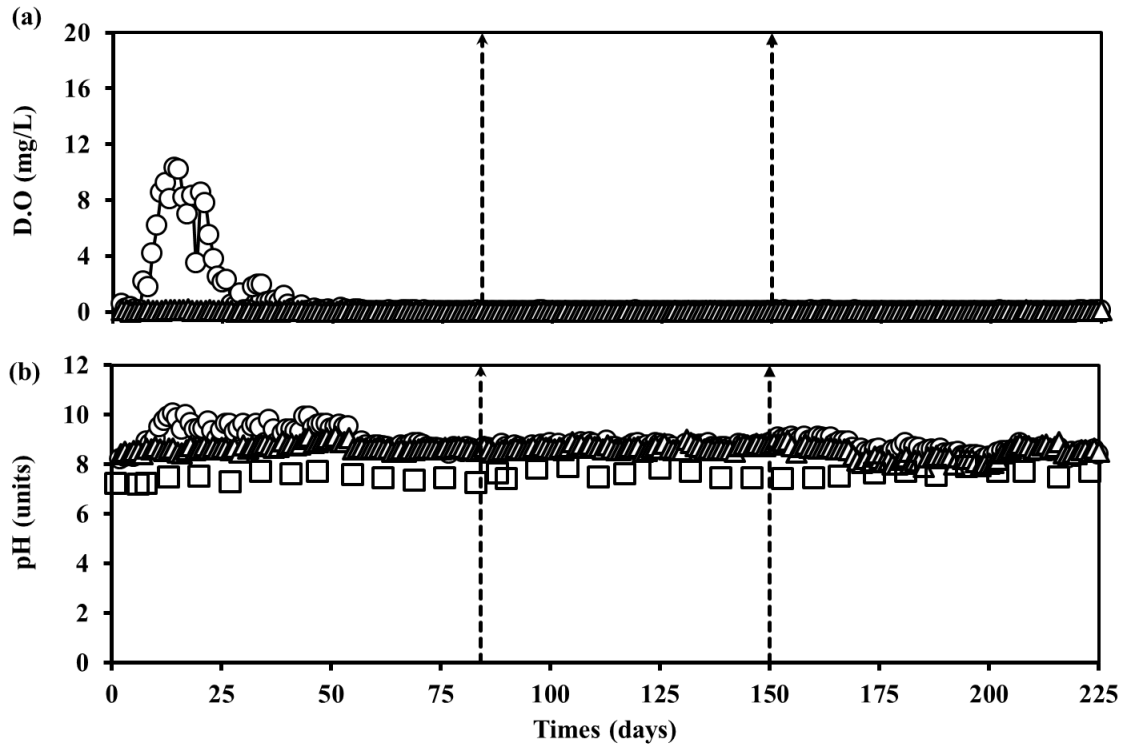


Figure S6. Time course of the dissolved oxygen concentration (**a**) and pH (**b**) in PWW (□), PBR-AB (○) and PBR-PBB (Δ).

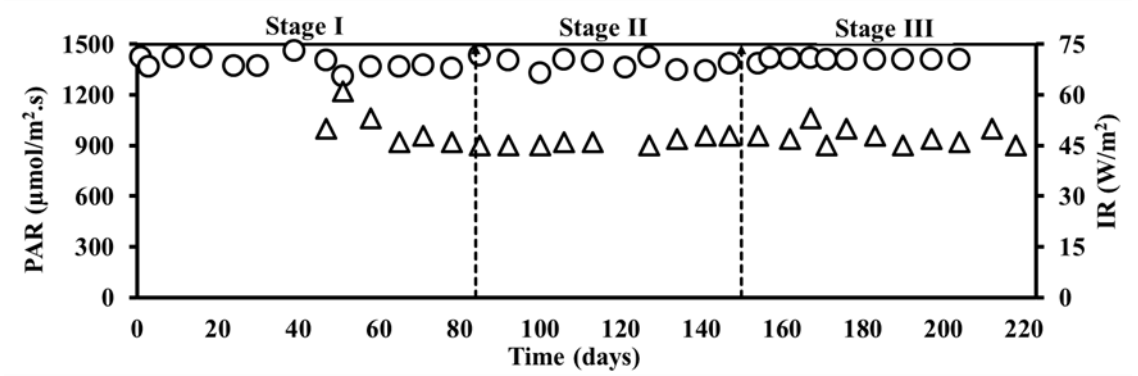


Figure S7. Time course of the average PAR and IR in PBR-AB (○) and PBR-PBB (Δ), respectively.

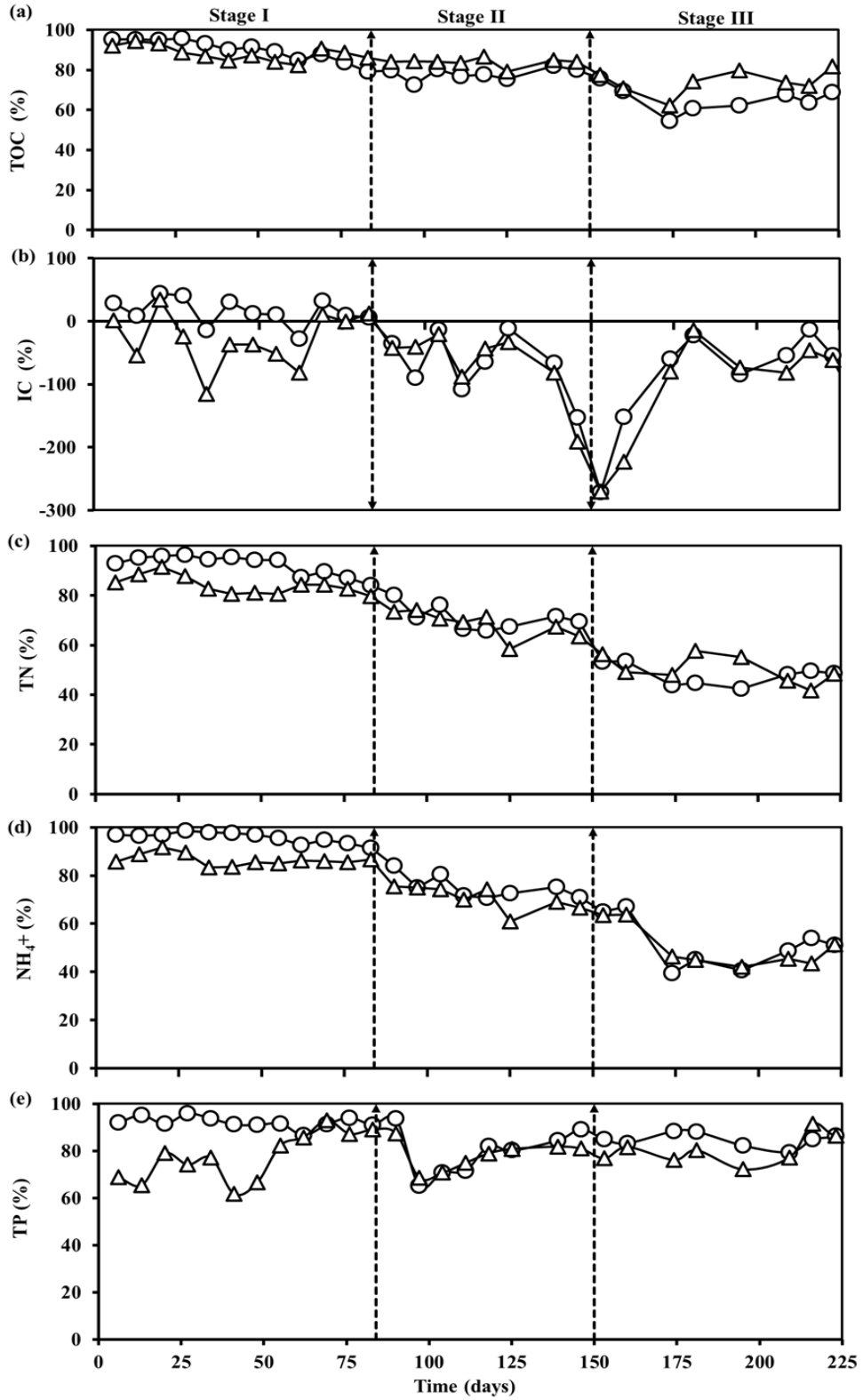


Figure S8. Time course of the removal efficiencies of TOC (a), IC (b), TN (c), N-NH_4^+ (d) and TP (e) in PBR-AB (○) and PBR-PBB (Δ). Vertical dashed lines separate the different operational stages evaluated.

Table S1. Operational parameters and photobioreactors dimensions in Stage I.

PBR	**HRT (d)**	**Q *feed* (L/d)**	**Q *eff* (L/d)**	**Evaporation rate (%)**	**V PBR (mL)**	**Height PBR (cm)**	**Area PBR (m^2)**
AB	10.6	0.282	0.079	72	3000	15	0.020
PPB			0.124	56			

PBR: Photobioreactor
AB: Algal-bacterial
PPB: Purple phototrophic bacteria

Table S2. Mass balance calculations of PWW treatment under steady state conditions in PBR-AB and PBR-PPB in Stage I.

Parameters	**PWW**	**PBR-AB**	**PBR-PPB**
TOC (mg/L)	592	309	181
TOC (mg/d) in		166.9	166.9
TOC (mg/d) out		24.4	22.5
% TOC RE		85	87
IC (mg/L)	57	169	142
IC (mg/d) in		16.1	16.1
IC (mg/d) out		13.3	17.6
% IC RE		17	-10
TN (mg/L)	160	68	65
TN (mg/d) in		45.1	45.1
TN (mg/d) out		5.4	8.1
% TN RE		88	82
Nitrite (mg/L)		< 0.5	< 0.5
Nitrate (mg/L)		< 0.5	< 0.5
TP (mg/L)	5.20	1.61	1.35
TP (mg/d) in		1.47	1.47
TP (mg/d) out		0.13	0.17
% TP RE		91	89
Zinc (mg/L)	0.85	0.07	0.015
Zn (mg/d) in		0.24	0.24
Zn (mg/d) out		0.006	0.002
% Zn RE		98	99
TSS (mg/L)	183	2640	873
Productivity (g/m2/d)		10.42	5.42
C %		44.8	50.7
C (mg/d) biomass		93	55
% Carbon recovered		64	38
N %		6.97	8.71
N (mg/d) biomass		14.53	9.43
% Nitrogen recovered		37	25
P%		0.62	0.72
P (mg/d) biomass		1.29	0.78
% Phosphorus recovered		97	60

Table S3. Operational parameters and photobioreactors dimensions in Stage II.

PBR	**HRT (d)**	**Q *feed* (L/d)**	**Q *eff* (L/d)**	**Evaporation rate (%)**	**V PBR (mL)**	**Height PBR (cm)**	**Area PBR (m^2)**
AB	7.6	0.396	0.230	42	3000	15	0.020
PPB			0.253	36			

PBR: Photobioreactor
AB: Algal-bacterial
PPB: Purple phototrophic bacteria

Table S4. Mass balance calculations of PWW treatment under steady state conditions in PBR-AB and PBR-PPB in Stage II.

Parameter	PWW	PBR-AB	PBR-PPB
TOC (mg/L)	566	199	136
TOC (mg/d) in		224.1	224.1
TOC (mg/d) out		45.7	34.5
% TOC RE		80	85
IC (mg/L)	54	144	137
IC (mg/d) in		21.4	21.4
IC (mg/d) out		33.1	34.7
% IC RE		-55	-62
TN (mg/L)	162	85	84
TN (mg/d) in		64.2	64.2
TN (mg/d) out		19.5	21.3
% TN RE		70	67
Nitrite (mg/L)		< 0.5	< 0.5
Nitrate (mg/L)		< 0.5	< 0.5
TP (mg/L)	6.00	1.62	1.75
TP (mg/d) in		2.38	2.38
TP (mg/d) out		0.37	0.44
% TP RE		84	81
Zinc (mg/L)	0.78	0.2	0.12
Zn (mg/d) in		0.31	0.31
Zn (mg/d) out		0.05	0.03
% Zn RE		85	90
TSS (mg/L)	263	1005	853
Productivity (g/m2/d)		12	11
C %		52	53
C (mg/d) biomass		121	115
% Carbon recovered		72	65
N %		9.08	8.72
N (mg/d) biomass		20.96	18.85
% Nitrogen recovered		47	44
P%		0.77	0.72
P (mg/d) biomass		1.78	1.56
% Phosphorus recovered		89	81

Table S5. Operational parameters and photobioreactors dimensions in Stage III.

PBR	**HRT (d)**	**Q *feed* (L/d)**	**Q *eff* (L/d)**	**Evaporation (%)**	**V PBR (mL)**	**Height PBR (cm)**	**Area PBR (m^2)**
AB	4.1	0.731	0.577	21	3000	15	0.020
PPB			0.599	18			

PBR: Photobioreactor
AB: Algal-bacterial
PPB: Purple phototrophic bacteria

Table S6. Mass balance calculations of PWW treatment under steady state conditions in PBR-AB and PBR-PPB in Stage III.

Parameter	PWW	PBR-AB	PBR-PPB
TOC (mg/L)	564	246	156
TOC (mg/d) in		412.3	412.3
TOC (mg/d) out		142.1	93.5
% TOC RE		66	77
IC (mg/L)	62	117	122
IC (mg/d) in		45.3	45.3
IC (mg/d) out		67.6	73.1
% IC RE		-49	-61
TN (mg/L)	176	118	110
TN (mg/d) in		128.7	128.7
TN (mg/d) out		68.1	65.9
% TN RE		47	49
Nitrite (mg/L)		< 0.5	< 0.5
Nitrate (mg/L)		< 0.5	< 0.5
TP (mg/L)	5.74	1.22	1.22
TP (mg/d) in		4.20	4.20
TP (mg/d) out		0.70	0.73
% TP RE		83	83
Zinc (mg/L)	0.69	0.08	0.06
Zn (mg/d) in		0.50	0.50
Zn (mg/d) out		0.05	0.04
% Zn RE		91	93
TSS (mg/L)	265	638	553
Productivity (g/m2/d)		18	17
C %		47	51
C (mg/d) biomass		173	169
% Carbon recovered		70	58
N %		6.55	8.74
N (mg/d) biomass		24.13	28.97
% Nitrogen recovered		40	46
P%		0.48	0.64
P (mg/d) biomass		1.77	2.12
% Phosphorus recovered		51	61

Table S7. Relative abundance (%) of the taxonomic composition for algal-bacterial (PBR-AB) and purple phototrophic bacteria (PBR-PPB) photobioreactors.

Phylum	Class	Order	Family	Genus	PBR-AB (%)				PBR-PPB (%)			
					Inoc.	SI	SII	SIII	Inoc.	SI	SII	SIII
Acidobacteria	*Acidobacteriia*	*Solibacterales*	*Solibacteraceae* (Subgroup 3)	*Paludibaculum*						0.5	2.5	1.5
Actinobacteria	*Coriobacteriia*	OPB41	uncultured *Actinobacteridae bacterium*	uncultured *Actinobacteridae bacterium*		1.3	3.4					
Chloroflexi	*Anaerolineae*	*Anaerolineales*	*Anaerolineaceae*	*Anaerolinea*			0.7	1.5			1.7	0.9
			Anaerolineaceae	*uncultured*		11.6	10.6	1.6			2.1	4.5
		SBR1031	A4b	uncultured *gamma proteobacterium*		1.6						
	Chloroflexia	*Chloroflexales*	*Chloroflexaceae*	*Chloronema*				4.3				
			Chloroflexaceae	__				1.8				
Cyanobacteria	*Oxyphotobacteria*	*Chloroplast*	*Tetradesmus obliquus*	*Tetradesmus obliquus*		2						
			__	__	26.9	23.7	3.1	13.2				
Epsilonbacteraeota	*Campylobacteria*	*Campylobacterales*	*Arcobacteraceae*	*Arcobacter*		18.8	8.2	10.7		46.6	19.9	19.8
Firmicutes	*BRH-c20a*	*uncultured bacterium mle1-9*	uncultured *bacterium mle1-9*	uncultured *bacterium mle1-9*				1.6				1.4
Firmicutes	*Clostridia*	*Clostridiales*	*Christensenellaceae*	*Christensenellaceae R-7 group*	1.0	0.8	6.2	3	1.5	0.8	7.2	3.5
			Family XI	*Sedimentibacter*				14.7			1.2	7.8
			Family XIII	*Anaerovorax*		0.9	13.5	3.6			2	3.1
				uncultured							1.2	6.6

Phylum	Class	Order	Family	Genus	PBR-AB (%)				PBR-PPB (%)			
					Inoc.	SI	SII	SIII	Inoc.	SI	SII	SIII
Firmicutes	*Clostridia*	*Clostridiale*	*Ruminococcaceae*	*Fastidiosipila*		1	8.3	5.1	2.1	4.5	11.3	9.3
				Ruminococcaceae UCG-013							1.6	
				Ruminococcaceae UCG-014			1				1.7	
				[Eubacterium] coprostanoligenes group		1.2	1.1	0.5				
				__		0.7	1.2	1.1			1.1	2.2
		DTU014	uncultured *Selenomonadales bacterium*	uncultured *Selenomonadales bacterium*			6.9	14.2			2.1	12.8
Patescibacteria	*Parcubacteria*	*Candidatus Kaiserbacteria*	*Candidatus Kaiserbacteria bacterium* RIFOXYB1_FULL_46_14	*Candidatus Kaiserbacteria bacterium RIFOXYB1_FULL_46_14*				1			4.1	
	Saccharimonadia	*Saccharimonadales*	*Saccharimonadaceae*	__							1.4	
	WS6 (Dojkabacteria)	uncultured *bacterium*	uncultured *bacterium*	uncultured *bacterium*		5.5	0.6			4.5	1	

Phylum	Class	Order	Family	Genus	PBR-AB (%)				PBR-PPB (%)			
					Inoc.	SI	SII	SIII	Inoc.	SI	SII	SIII
Proteobacteria	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Caulobacter*	3.7							
		Rhizobiales	*Beijerinckiaceae*	*Methylocystis*			1.9			0.7	9	3.1
			Rhizobiaceae	__		1.3				10.2	1	
			Xanthobacteraceae	*Rhodoplanes*			18.7	1.5		9.2	11.4	7.5
				Rhodopseudomonas					81.7	1.7		
		Rhodobacterales	*Rhodobacteraceae*	*Paracoccus*			1					
				Rhodobacter				6.3				
				__		7.2		1.5		4.6	1	
		Rhodospirillales	*Magnetospirillaceae*	*Magnetospirillum*				1.6				1.6
		Sphingomonadales	*Sphingomonadaceae*	*Blastomonas*	49.7							
				Novosphingobium	6.3	10.6			1	4.2	2.3	
				Sphingomonas	7.4				1			
Synergistetes	*Synergistia*	*Synergistales*	*Synergistaceae*	*Aminobacterium*					5.3			
				Others	5	11.8	13.5	11.1	7.3	12.6	13.5	14.4
				Number of cells	**34903**	**18964**	**13683**	**28884**	**44503**	**29644**	**19355**	**14765**

Conclusions and future work

Several photobioreactor configurations were evaluated during wastewater treatment under indoor and outdoor conditions, along with several photosynthetic microorganisms. In addition, state-of-the-art operational strategies such as CO_2 supplementation, biomass settling and recycling, and wastewater dilution, were tested to boost pollutant biodegradation and biomass productivity during wastewater treatment. Overall, the results herein obtained confirmed the potential of photosynthetic processes for domestic and livestock wastewater treatment as a cost-effective and enviromental friendly technology platforms.

Firstly, a novel anoxic-aerobic algal-bacterial photobioreactor with biomass settling and recycling was evaluated using real domestic wastewater. This study was conducted to improve processes performance by overcoming the main limitation present in domestic wastewater as a result of its low C/N/P ratio (inorganic carbon). The results obtained in **Chapter 1** demonstrated that algal-bacterial symbiosis supported an efficient biodegradation of TOC and TN when carbon dioxide from biogas was supplemented. In this context, biogas supplementation avoided the need for pH control, and enhanced TOC and TN removals by promoting the activity of nitrifying bacteria up to complete ammonium oxidation. On the other hand, a stable and high biomass concentration was achieved by recirculation of the settled biomass, which also supported an effluent TSS concentration complying with EU regulations. Likewise, biomass settling and recycling also contibuted to the enrichment of dominant monoalgal cultures with good settling properties.

The long-term dominance of a specific microalga or cyanobacterium under indoor conditions treating piggery wastewater was evaluated in four open photobioreactors. Despite the optimal environmental conditions of light, temperature and pH (controlled via CO_2 addition) prevailing throughout the experiment, the results obtained in **Chapter 2** showed a difficulty to maintain a monoalgal culture in all photobioreactors. Interestingly, the highest biomass concentrations were recorded in the control photobioreactor at the end of the experiment as a result of the acclimation of native species. *Chlorella sp.* was dominant in R3 and R4, which highlighted the high tolerance of this microalga to organic pollution. Finally, an efficient PWW treatment occurred regardless of the microalgae species inoculated, with stripping identified as the main mechanism responsible for carbon and nitrogen removal.

A comparative study (**Chapter 3)** was carried to evaluate microalgae evolution and process performance under indoor and outdoor conditions during the treatment of PWW. Four open photobioreactors were operated to elucidate the representativeness of the results obtained indoor under artificial irradiation. A comparable TOC removal performance was recorded regardless of the photobioreactor location. Higher TN removal efficiencies were recorded at lowest dilutions (×20) both indoors and outdoors. Stripping was the main mechanism responsible for TOC and TN removal during the treatment of 10× PWW. *Chlorella vulgaris* became dominant in all photobioreactors regardless of the environmental conditions and PWW dilution, which confirmed the high pollution-tolerance of this microalga species. The highest microalgae concentrations were recorded during the treatment of 20× PWW regardless of the environmental conditions likely due to the lower toxicity of this effluent. On the other hand, the molecular analysis of the bacterial populations revealed a high dominance of the *Proteobacteria* phylum in all photobioreactors, and the key influence of temperature and irradiation on the final bacterial population structure. The results herein obtained demonstrated for the first time that neither pollutant removal nor the structure of microalgae and bacterial communities under indoor conditions can be directly extrapolated to outdoors photobioreactors.

Finally, the potential of a microalgal-bacterial consortium and purple phototrophic bacteria was comparatively evaluated during the continuous treatment of PWW under indoor conditions in two open photobioreactors. In addition, the influence of PWW dilution on the biodegradation performance of these photosynthetic consortia was assessed batchwise (**Chapter 4**). Overall, the results revealed a similar treatment performance of both photosynthetic microorganisms in terms of carbon, nutrients and zinc removal. PBR-PPB exhibited a slightly better capacity to remove organic matter (which was not observed during batch PWW treatment), while a superior carbon and nutrient recovery was recorded in PBR-AB. Assimilation was the main mechanism responsible for carbon removal (except in PBR-PPB at high HRT), while stripping governed N removal in both PBRs. Phosphorus assimilation and Zn biosorption were likely the main mechanisms responsible for the removal of these inorganic pollutants based on the moderate pH values prevailing in both PBRs. Higher biomass productivities were recorded in PBR-AB mediated by microalgae CO_2 recovery. The stepwise decrease in HRT, rather than the type of illumination used, caused significant changes in the structure of microalgae and bacterial population. The batch biodegradation tests

conducted at multiple PWW dilutions (5, 10 and 15%) confirmed the slightly better performance of algal-bacterial systems regardless of PWW dilution.

Based on the outcomes and limitations found in this study, further research on photosynthetic biodegradation should focus on:

1. Use of pollution tolerant microorganisms in order to increase pollutant removal and enhance biomass production based on the findings observed in **Chapter 2**.

2. The scale-up of photobioreactors under outdoor conditions, which will provide more representative information about pollutant removal performance and biomass production based on the results obtained in **Chapter 1 and 3**.

3. Evaluation of alternative photobioreactors configuration in order to avoid high evaporation rates and their negative influence on the quality of the treated effluents based on the observations in **Chapter 2 and 3**.

4. Optimization of the cultivation conditions of PPB in order to identify the full potential of these microorganisms for PWW treatment based on the results recorded in **Chapter 4**.

About the author

Biography

Dimas Alberto García Guzmán (Masaya, Nicaragua, 1973)

Dimas A. García studied Chemistry at Universidad Nacional Autónoma de Nicaragua UNAN-Managua (1994-1999). In 2010, Dimas was awarded with a scholarship to study a Master's Degree in Water Science at Centro para la Investigación en Recursos Acuáticos de Nicaragua (CIRA/UNAN-Managua). His Master's thesis was entitled "Zonificación Hídrica de la microcuenca del río La Trinidad, en la subcuenca del Río Viejo, Estelí, Nicaragua". In July 2014, Dimas was awarded with a scholarship from the **EURICA** project (**Erasmus Mundus Action 2, Strand 1, Lot 15, Grant Agreement number 2013-2587**) of the European Union to study a doctorate in Chemical Engineering and Environmental Technology at Valladolid University, Spain.

In September 2014, Dimas joined the Biological Gas Treatment and Microalgae Technology Research Group headed by Professor Raúl Muñoz Torre within the Environmental Technology Group of the Department of Chemical Engineering and Environmental Technology. His PhD thesis was focused on the development of innovative biotechnological processes for the treatment of domestic and agroindustrial wastewater using photobioreactors (September 2014 - September 2018).

Dimas A. García has been working at CIRA/UNAN-Managua since July 2000 as a lecturer on Limnology and Hydrogeochemestry. In addition, Dimas has an extensive experience in monitoring water resources such as rivers and lakes, and industrial effluents and wastewater treatment plants. Dimas has been an internal auditor in analytical quality management at CIRA/UNAN-Managua.

Publications in International Indexed Journals

García D., de Godos, I., Dominguez, C., Turiel, S., Bolado, S., Muñoz, R., 2018. ***A systematic comparison of the potential of a microalgal-bacterial consortium and purple phototrophic bacteria for the continuous treatment of piggery wastewater***. Submitted for puplication to Bioresource Technology.

Dhaouefi, Z., Toledo-Cervantes, A., **García, D.**, Bedoui, A., Ghedira, K., Chekir-Ghedira, L.; Muñoz, R., 2018. ***Assessing textile wastewater treatment in an anoxic-aerobic photobioreactor and the potential of the treated water for irrigation***. Algal Research. 29, 170 - 178. doi:10.1016/j.algal.2017.11.032

García, D., Posadas, E., Grajera, C., Blanco, S., Martínez, S., Acién, G., García-Encina, P., Bolado, S., Muñoz, R., 2017. ***Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions***. Bioresource Technology. 245 (Pt A), 483 - 490. doi.10.1016/j.biortech.2017.08.135

García, D., Posadas, E., Blanco, S., Acién, G., García-Encina, P., Bolado, S., Muñoz, R., 2018. ***Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors***. Bioresource Technology. 248 (Pt B), 120 - 126. doi:10.1016/j.biortech.2017.06.079

García, D., Alcántara, C., Blanco, S., Pérez, R., Bolado, S., Muñoz, R., 2017. ***Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading***. Chemical Engineering Journal. 313, 424 - 434. doi:10.1016/j.cej.2016.12.054

Alcántara, C., Domínguez, J.M., **García, D**., Blanco, S., Pérez, R., García-Encina, P.A., Muñoz, R., 2015. ***Evaluation of wastewater treatment in a novel anoxic-aerobic algal-bacterial photobioreactor with biomass recycling through carbon and nitrogen mass balances***. Bioresource Technology. 191, 173 - 186. doi:10.1016/j.biortech.2015.04.125

Conferences

1. López R, Pérez S, Bolado S, Irustra R, Ortiz S, Marín D, Páramo S, Hernando N, **García D**, Posadas E, Pérez N, Díaz A, Muñoz R, García-Encina P. "***Tratamiento de contaminantes emergentes en sistemas de tratamiento de aguas residuales y lodos*"**. XIII reunión de la Mesa Española de Tratamiento de Aguas Residuales 2018. 18 - 20 junio 2018, León, España. (Oral Presentation)

2. Dhaouefi Z, Toledo-Cervantes A, **García D**, Bedoui Ahmed, Ghedira K, Muñoz R, Chekir-Guedira L. "***Assessment of Algal-bacterial symbiosys in the treatment of textile wastewater***". 7èmes Journées Internationales Scientifiques de l'ATT «Exposition aux faibles doses et aux mélanges, Interaction et modulation des effets» 27 - 29thApril 2018, Monastir, Tunisia. (Oral Presentation)

3. Rodero R, Ángeles R, **García D**, Lebrero R, García P, Muñoz R. **"*Procesos de microalgas como plataforma para la mejora de la eficiencia energética en EDARs*"**. Jornada NOVEDAR. 3 de abril 2017, Madrid, España. (Oral Presentation)

4. **García D**, Bolado S, Muñoz R. "***Photosynthetic biodegradation of domestic and agroindustrial wastewater***". XI Reunión de Jóvenes Investigadores Iberoamericanos de la UVa. 13 de abril del 2018. Valladolid, España. (Poster)

5. **García D**, Posadas E, Blanco S, Acién G, García-Encina P, Bolado S, Muñoz R. "***Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal- bacterial photobioreactors"***. 15th International Conference on Environmental Science and Technology (CEST). 31st August – September 2nd 2017, Rhodes, Greece (Oral Presentation).

6. **García D**, Alcántara C, Blanco S, Pérez R, Bolado S, Muñoz R. "***Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic aerobic photobioreactor coupled with biogas upgrading*"**. X Reunión de Jóvenes Investigadores Iberoamericanos de la Uva. 31 de marzo del 2017, Valladolid, España (Poster).

7. Alcántara C, Domínguez J, **García D**, Blanco S, Pérez R, García-Encina P.A, Muñoz R. "***Wastewater Treatment In A Novel Anoxic-aerobic Algal-bacterial Photobioreactor With Biomass Recycling"***. 3rd IWA Specialized International Conference Ecotechnologies for Wastewater Treatment 2016 (ecoSTP16). 27 - 30 June. Cambridge, UK (Oral presentation*).*

8. Bolado S, Martín J, Lebrero R, Pérez S, **García D**, Marín D, Lorenzo A, García P.A, Muñoz R. "***Tratamiento y valorización de aguas residuales mediante microalgas"***. XII reunión de la Mesa Española de Tratamiento de Aguas Residuales. 20 - 22 junio 2016, Madrid, España. (Oral Presentation)

9. Lorenzo A, Martín J, **García D**, Bolado S**. "*Influencia de las condiciones de producción de biomasa microalgal procedente de aguas residuales en la liberación de azúcares mediante hidrólisis enzimática"***. Bio.Iberoamérica 2016: Biotecnología integrando continentes. 5 - 8 June 2016. Salamanca, España. (Poster)

10. **García D**, Alcántara C, Blanco S, Pérez R, Bolado S, Muñoz R. "***Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic aerobic photobioreactor coupled with biogas upgrading"***. 10th International Society for Environmental Biotechnology (ISEB) Conference. 1 - 3 June 2016, Barcelona, Spain (Oral presentation).

Supervision

Supervision of Master Thesis

❖ Keira Acosta (diciembre 2017 - octubre 2018). "Caracterización limnológica y de la calidad de agua del lago de Yojoa, Honduras". Universidad Nacional Autónoma de Nicaragua (CIRA/UNAN-Managua).

Co-supervision of Final Degree Thesis

❖ Christian Domínguez (julio 2017 - julio 2018). "Tratamiento biológico de aguas residuales de origen porcino. Estudio comparativo entre consorcio algas-bacterias y bacterias púrpuras fotosintéticas del no sulfuro". Universidad de Valladolid (España).

Co-supervision of Master Thesis

❖ Carlos Grajeda Cárdenas (abril 2016 - julio 2016). "Eliminación fotosintética de materia orgánica y nutrientes provenientes de Purín de cerdo utilizando diferentes tipos de iluminación: LED y luz solar". Universidad de Valladolid (España).