Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

Optimization of biogas upgrading in algal-bacterial photobioreactors at pilot and demo scale

Presentada por **María del Rosario Rodero Raya**
para optar al grado de
Doctora por la Universidad de Valladolid

Dirigida por:
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Certifican que:

MARÍA DEL ROSARIO RODERO RAYA ha realizado bajo su dirección el trabajo "*Optimization of biogas upgrading in algal-bacterial photobioreactors at pilot and demo scale*", en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Escuela de Ingenierías Industriales de la Universidad de Valladolid. Considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresan su conformidad con dicha presentación.

Valladolid, a _____ de_____ de 2020

Fdo. Raúl Muñoz Torre

Fdo. Raquel Lebrero Fernández

Universidad de Valladolid

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Reunido el tribunal que ha juzgado la Tesis Doctoral titulada *"Optimization of biogas upgrading in algal-bacterial photobioreactors at pilot and demo scale"* presentada por María del Rosario Rodero Raya y en cumplimiento con lo establecido por el Real Decreto 99/2011 de 28 de enero de 2011 acuerda conceder por_____ la calificación de _____.

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Resumen

El biogás, principal subproducto de la digestión anaerobia de residuos sólidos o fangos del tratamiento de aguas residuales, constituye una fuente de bioenergía con alto potencial para reducir parcialmente el actual consumo de combustibles fósiles. A pesar de este potencial, su aprovechamiento como sustituto renovable del gas natural aún presenta importantes limitaciones, entre ellas la presencia de contaminantes como CO_2 y H_2S. Una disminución en el contenido de CO_2 del biogás resultará en un aumento del contenido energético de éste, una disminución de los costes de transporte, así como en menores emisiones de gases de efecto invernadero (GEIs) asociadas a su combustión. Del mismo modo, la eliminación del H_2S es decisiva al ser altamente corrosivo, tóxico y generar molestias por malos olores. El mercado de tecnologías de limpieza (*upgrading*) de biogás para su conversión a biometano está dominado en la actualidad por tecnologías físico-químicas, que presentan tanto altos costes de operación e inversión como impactos ambientales negativos. Además, no existe todavía en el mercado ninguna tecnología capaz de eliminar simultáneamente el CO_2 y H_2S del biogás.

En este contexto, la intensificación de la simbiosis entre microalgas y bacterias heterótrofas/quimioautótrofas en fotobiorreactores representa una plataforma tecnológica muy novedosa para la eliminación simultánea de CO_2 y H_2S del biogás. En estos sistemas, las microalgas usan la energía proveniente de la luz solar para fijar el CO_2 del biogás vía fotosíntesis, con la consiguiente generación de oxígeno. Este oxígeno generado *in-situ* será empleado por bacterias quimioautótrofas para la oxidación de H_2S a sulfato. Esta tecnología permite además una recuperación de nutrientes de aguas residuales o centrados en forma de biomasa que podría utilizarse como biofertilizante, mejorando así la sostenibilidad ambiental y económica del proceso. Sin embargo, esta tecnología aún requiere una mayor optimización para superar las limitaciones técnicas (por ejemplo, transferencia de masa de CO_2 limitada, baja eficiencia de sedimentación para la separación de biomasa) y el escalado del proceso es necesario para mejorar su aceptación por el sector industrial e impulsar su implementación generalizada a gran escala. Por tanto, esta tesis tiene como objetivo evaluar sistemáticamente la influencia de parámetros ambientales y operativos en el rendimiento del upgrading fotosintético de biogás, y desarrollar un sistema de control para optimizar la operatividad del proceso a escala piloto y semi-industrial antes de su exitosa implementación a escala industrial.

Para ello, en el **Capítulo 3** se evaluó la influencia de la alcalinidad (1500, 500 y 100 mg de carbono inorgánico L^{-1}) y la temperatura (12 y 35°C) del caldo de cultivo en la calidad del biometano en un fotobiorreactor abierto de lagunaje de 180 L interconectado a una columna de absorción de 2.5 L mediante recirculación del caldo de cultivo previamente sedimentado. En este estudio, la alcalinidad del caldo de cultivo se identificó como un parámetro ambiental clave para fomentar una alta transferencia de masa de CO_2 y H_2S en la columna de absorción y, en consecuencia, obtener una alta pureza de CH_4 en el biometano. Por otro lado, la temperatura tuvo un efecto insignificante sobre la calidad del biometano a alcalinidades medias-altas, mientras que, a alcalinidades bajas, la calidad del biometano mejoró cuando se disminuyó la temperatura.

Los resultados obtenidos en el capítulo anterior destacaron el papel clave del pH durante la limpieza fotosintética del biogás y la necesidad de una alta alcalinidad en el caldo de cultivo para mantener un pH alto a lo largo de la columna de absorción. Sin embargo, una alta alcalinidad en el caldo de cultivo podría ejercer un impacto negativo en la actividad fotosintética y contribuir a intensificar la desorción de CO_2 a la atmósfera. En este sentido, se evaluó la operación a largo plazo del sistema de upgrading de biogás trabajando a altas concentraciones de carbono inorgánico (**Capítulo 4**). Aunque las concentraciones de carbono inorgánico superiores a 2400 mg C L^{-1} mejoraron la eficacia y la robustez del proceso en términos de calidad de biometano, estas altas concentraciones de carbono inorgánico en el caldo de cultivo contribuyeron a aumentar la cantidad de CO_2 emitido a la atmósfera y a disminuir la actividad fotosintética, reduciendo así el beneficio medioambiental de esta tecnología. Además se evaluó el efecto de la concentración de biomasa en el fotobiorreactor abierto sobre la operación del proceso, ejerciendo una alta concentración de biomasa un impacto negativo tanto en la transferencia de masa del CO_2 entre el gas y el líquido en la columna de absorción como en la productividad de la biomasa.

En el **Capítulo 5**, el escalado del proceso de *upgrading* del biogás acoplado al tratamiento de aguas residuales se realizó en un fotobiorreactor abierto de 9.6 m^3 interconectado a una columna de absorción de 150 L mediante la recirculación del caldo de cultivo de un sedimentador de 7 m^3. El proceso se llevó a cabo en ambiente exterior. La influencia de la relación líquido/biogás (L/G = 1.2, 2.1 y 3.5) y del caudal de biogás (274, 370 y 459 L h^{-1}) en la columna de absorción, el tiempo de retención hidráulico

(TRH) en el fotobiorreactor y el tipo de fuente de nutrientes (aguas residuales domésticas o digestato) sobre la calidad del biometano fueron evaluados. A pesar de que la relación L/G más alta consiguió las eliminaciones más altas de CO_2 y H_2S, el contenido de CH_4 en el biometano estaba limitado por la desorción asociada de N_2 y O_2. No se observó una influencia significativa del caudal de biogás y TRH en el fotobiorreactor sobre el rendimiento del proceso de *upgrading*, mientras que el tipo de fuente de nutrientes se identificó como un parámetro operativo clave: el uso de centrado mejoró la eliminación de CO_2 y H_2S como resultado de su alto pH y alcalinidad. Este trabajo constituyó la primera validación a escala semi-industrial de un proceso de algas y bacterias para la eliminación simultánea de CO_2 y H_2S del biogás acoplado al tratamiento de aguas residuales en condiciones exteriores.

Para la comercialización del biometano se requiere una calidad uniforme que permita su inyección en redes de gas natural o su uso como combustible para vehículos, independientemente de las condiciones ambientales o los posibles fallos operativos. Para ello, el diseño y validación de un sistema de control para la tecnología de limpieza de biogás fotosintético se llevó a cabo en el **Capítulo 6** en un sistema experimental similar al de los Capítulos 3 y 4. El caudal del líquido de recirculación, e indirectamente la relación L/G en la columna de absorción, fue seleccionado como variable manipulada con el fin de controlar el contenido de CO_2 y O_2 del biometano, y por tanto cumplir con los requisitos para su uso como sustituto del gas natural. La estrategia de control desarrollada fue capaz de mantener la concentración de CO_2 por debajo del valor de consigna (2.5%), partiendo de una concentración inicial de 29.5% CO_2 en el biogás, ante cualquier alteración en el caudal de biogás. Además, se obtuvo un contenido de O_2 inferior al 1% y contenidos despreciables de H_2S en el biometano a diferentes concentraciones de carbono inorgánico (1500, 500 y 100 mg C L^{-1}) y temperaturas (15 y 35°C) en el caldo de cultivo a un pH de 10. Sin embargo, la disminución del pH del caldo de cultivo hasta 8.5 conllevó una baja transferencia de masa de CO_2 que resultó en la necesidad de operar a altos caudales de líquido. En estas condiciones, se produjo un aumento significativo de las cantidades de O_2 transferidas del líquido de recirculación al biometano y, en consecuencia, un contenido de O_2> 1% en el biometano.

Con base en estos resultados prometedores obtenidos, la validación de la estrategia de control se llevó a cabo en un fotobiorreactor abierto a escala semi-industrial (**Capítulo 7**). El sistema de control aseguró una calidad de biometano constante (CH_4> 95%, CO_2

<2%, O_2 <1% y H_2S=0) independientemente del pH en el caldo de cultivo (9.05-9.50) o variaciones en el caudal de biogás de 143 a 420 L h^{-1}. Además, el sistema de control restauró la calidad del biometano después de fallos en el suministro de biogás o del líquido de recirculación. Estos resultados confirmaron la eficacia de la estrategia de control para evitar los efectos adversos en el funcionamiento del sistema fotosintético de *upgrading* de biogás a pesar de las inevitables fluctuaciones de las condiciones ambientales o fallos operativos.

Finalmente, en el **Capítulo 8** se evaluó el uso de la floculación para mejorar el cosechado de la biomasa de microalgas y bacterias. Esto permitiría utilizar el caldo de cultivo libre de biomasa como líquido de lavado en la columna de absorción, aumentando así la transferencia de masa de CO_2 entre el líquido y el gas como se demostró en el Capítulo 4. En esta investigación, se probó la eficiencia de diferentes floculantes para el cosechado de la biomasa en condiciones de alta alcalinidad y pH, resultando sólo Zetag 8125 (un floculante sintético con base de acrilamida) y nanocristales de celulosa modificados catiónicamente (CNCs) en eficiencias de floculación > 90% sin efecto perjudicial para el cultivo cuando se recicla el sobrenadante. Además, se demostró que el filtrado a través de una malla de nylon con un tamaño de poro de 180 µm después de la floculación es una alternativa prometedora a la sedimentación por gravedad como etapa de separación a continuación de la floculación.

Los resultados obtenidos en la presente tesis conducen a una mejora en el rendimiento del proceso de *upgrading* de biogás basado en procesos de algas y bacterias, y un primer paso hacia la industrialización de esta biotecnología. Su comercialización contribuiría al aumento en el uso de biogás generado a partir de residuos orgánicos como sustituto del gas natural vía *upgrading* de una manera rentable y ambientalmente sostenible.

Abstract

Biogas, the main byproduct from the anaerobic digestion of organic solid waste or sludge from wastewater treatment, constitutes a potential bioenergy source able to reduce the current consumption of fossil fuels. However, its use as a renewable substitute for natural gas still presents limitations like the presence of pollutants such as CO_2 and H_2S. A decrease in the CO_2 concentration of biogas increases its energy content and decreases transportation costs and the greenhouse gas (GHG) emissions associated to its combustion. Likewise, the removal of H_2S is a requirement since it is highly corrosive, toxic and generates odor nuisance. The biogas upgrading market is currently dominated by physical-chemical technologies, which present both high operating and investment costs and negative environmental impacts. Furthermore, there is still no commercial technology able to remove CO_2 and H_2S simultaneously.

In this context, the intensification of the symbiosis between microalgae and heterotrophic/chemoautotrophic bacteria in photobioreactors represents an innovative platform for the simultaneous removal of CO_2 and H_2S from biogas. In these systems, microalgae use the light energy to fix the CO_2 via photosynthesis with the subsequent generation of oxygen. This oxygen generated *in-situ* is used by sulfur oxidizing bacteria for the oxidation of H_2S to sulfate. This biotechnology can also support the recovery of nutrients from wastewaters or centrates in the form of biomass, which could be used as biofertilizer, thus improving the environmental and economic sustainability of this process. Nevertheless, this technology still requires more optimization to overcome the current technical constraints (e.g. limited CO_2 gas-liquid mass transfer, poor efficiency of settling for biomass separation) and a process scale-up must be conducted in order to increase the acceptance of this technology in the industrial sector and boost its widespread full-scale implementation. Therefore, this thesis aims at evaluating the influence of environmental and operational parameters on photosynthetic biogas upgrading performance and developing a control system to optimize the performance of the process at pilot and semi-industrial scale prior to a successful industrial implementation.

For this purpose, in **Chapter 3** the influence on biomethane quality of the alkalinity (1500, 500 and 100 mg inorganic carbon L^{-1}) and temperature (12 and 35°C) of the cultivation broth was assessed in a 180 L high rate algal pond (HRAP) interconnected to a 2.5 L absorption column via settled broth recirculation. In this study, the alkalinity of

the cultivation broth was identified as a key environmental parameter to support a high CO_2 and H_2S gas-liquid mass transfer in the absorption column and consequently, obtaining high CH_4 purity in the upgraded biogas. On the other hand, a negligible effect of the temperature on the quality of the upgraded biogas was recorded at high-medium alkalinities, while low temperature improved biomethane quality at a low alkalinity.

The results obtained in the previous chapter highlighted the key role of the pH during photosynthetic biogas upgrading and the need to maintain a high alkalinity in the cultivation broth to support a high pH along the absorption column. However, a high alkalinity in the cultivation broth could exert a negative impact on the photosynthetic activity and contribute to intensify CO_2 stripping to the atmosphere. In this regard, the long-term performance of photosynthetic biogas upgrading was evaluated under high inorganic carbon concentrations (**Chapter 4**). Although inorganic carbon concentrations higher than 2400 mg C L^{-1} can improve the effectiveness and robustness of the process in terms of biomethane quality, these high inorganic carbon concentrations in the cultivation broth entail an increase in the amount of CO_2 stripped to the atmosphere and decrease the photosynthetic activity, thus reducing the environmental benefit of this technology. Moreover, the effect of biomass concentration in the HRAP on process operation was also assessed, a high biomass concentration exerting a negative impact on both CO_2 gas-liquid mass transfer in the absorption column and biomass productivity.

In **Chapter 5**, the scale-up of photosynthetic biogas upgrading coupled to wastewater treatment was performed in an outdoors 9.6 m^3 HRAP interconnected to a 150 L absorption column via recirculation of the cultivation broth from a 7 m^3 settler. The influence of liquid to biogas ratio (L/G = 1.2, 2.1 and 3.5) and biogas flowrate (274, 370 and 459 L h^{-1}) in the absorption column, hydraulic retention time (HRT) in the HRAP and type of nutrient source (domestic wastewater *vs* centrate) on the quality of the biomethane was evaluated. Despite the highest L/G ratio supported the highest CO_2 and H_2S removals, CH_4 content in the biomethane was limited by the associated N_2 and O_2 desorption. No significant influence of biogas flowrate and HRT in the HRAP on process performance was observed, while the type of nutrient source was identified as a key operational parameter, the use of centrate enhancing CO_2 and H_2S removals as a result of its high pH and alkalinity. This work represented the first demo-scale validation of algal-bacterial processes devoted to the simultaneous removal of CO_2 and H_2S from biogas coupled to wastewater treatment under outdoor conditions.

A consistent biomethane quality for its injection into natural gas grids or its use as vehicle fuel regardless of environmental conditions or operational failures is required for biomethane commercialization. For this purpose, the design and evaluation of a control system for the photosynthetic biogas upgrading unit was successfully carried out in **Chapter 6** in a similar system than that used in Chapters 3 and 4. The recycling liquid flowrate, and indirectly the liquid to biogas (L/G) ratio in the absorption column, was selected as the manipulated variable in order to control the CO_2 and O_2 content of biomethane, and therefore comply with the requirements for its use as natural gas substitute. The control strategy developed was capable of maintaining the CO_2 concentration below the set point (2.5%) from a concentration of 29.5% CO_2 in the raw biogas under any disturbance in the biogas flowrate together with an O_2 content lower than 1% and negligible H_2S contents in the biomethane at different inorganic carbon concentrations (1500, 500 and 100 mg C L^{-1}) and temperatures (15 and 35°C) in the cultivation broth at a pH of 10. However, the decrease in the pH of the cultivation broth down to 8.5 involved a low CO_2 mass transfer resulting in high liquid flowrates, which led to large amounts of O_2 stripped from the recycling liquid to the biomethane, and consequently a biomethane O_2 content >1%.

Based on these promising results, the validation of the control strategy was further performed in a semi-industrial scale outdoors photobioreactor (**Chapter 7**). The control system was able to ensure a consistent biomethane quality (CH_4>95%, CO_2<2%, O_2<1% and no H_2S) regardless of the pH in the cultivation broth (9.05-9.50) and variations in the biogas flowrate from 143 to 420 L h^{-1}. Moreover, the control system restored the biomethane quality after a failure in the biogas or liquid supply. These results confirmed the effectiveness of the control strategy to avoid adverse effects on the biogas upgrading performance due to the inherent environmental fluctuations or operational failures in this technology.

Finally, the use of flocculation to enhance the microalgal-bacterial harvesting in order to use the biomass-free cultivation broth as scrubbing liquid in the absorption column and increase the CO_2 gas liquid-mass transfer as demonstrated in Chapter 4 was assessed in **Chapter 8**. In this research, the harvesting efficiency of different flocculants was tested under high alkalinity and pH conditions. Only Zetag 8125 (a synthetic acrylamide-based flocculant) and cationically modified cellulose nanocrystals (CNCs) resulted in flocculation efficiencies >90% with no detrimental effect to the culture when the

supernatant was recycled. In addition, screening with a nylon mesh of 180 µm pore size after flocculation was demonstrated to be a promising alternative to gravity settling as a separation step.

The results obtained in the present thesis improved biogas upgrading performance based on algal-bacterial processes and represented a first step in the scale up of this green technology towards industrialization. The commercialization of this technology would contribute to increase the use of biogas from waste resources as a natural gas substitute via upgrading in a cost-effective and environmentally sustainable way.

List of publications

The following publications are presented as part of the current thesis. All papers were published in international journals indexed in ISI Web of Knowledge.

Manuscript I. Rodero, M. del R., Posadas, E., Toledo-Cervantes, A., Lebrero, R., Muñoz, R., 2018. Influence of alkalinity and temperature on photosynthetic biogas upgrading efficiency in high rate algal ponds. Algal Res. 33, 284–290. doi:10.1016/j.algal.2018.06.001.

Manuscript II. Rodero, M. del R., Lebrero, R., Serrano, E., Lara, E., Arbib, Z., García-Encina, P.A., Muñoz, R., 2019. Technology validation of photosynthetic biogas upgrading in a semi-industrial scale algal-bacterial photobioreactor. Bioresour. Technol. 279, 43–49. doi:10.1016/j.biortech.2019.01.110.

Manuscript III. Rodero, M. del R., Carvajal, A., Castro, V., Navia, D., de Prada, C., Lebrero, R., Muñoz, R., 2019. Development of a control strategy to cope with biogas flowrate variations during photosynthetic biogas upgrading. Biomass and Bioenergy 131, 105414. doi:10.1016/j.biombioe.2019.105414.

Manuscript IV. Rodero, M. del R., Carvajal, A., Arbib, Z., Lara, E., de Prada, C., Lebrero, R., Muñoz, R., 2020. Performance evaluation of a control strategy for photosynthetic biogas upgrading in a semi-industrial scale photobioreactor. Bioresour. Technol. 307, 123207. doi:10.1016/j.biortech.2020.123207.

Manuscript V. Rodero, M. del R., Muñoz, R., Lebrero, R., Verfaillie, A., Blockx, J., Thielemans, W., Muylaert, K., Praveenkumar, R., 2020. Harvesting microalgal-bacterial biomass from biogas upgrading process and evaluating the impact of flocculants on their growth during repeated recycling of the spent medium. Algal Res. 48, 101915. doi:10.1016/j.algal.2020.101915.

Manuscript VI. Rodero, M. del R., Severi, C., Rocher-Rivas, R., Quijano, G., Muñoz, R., 2020 Long-term influence of high alkalinity on the performance of photosynthetic biogas upgrading. Fuel 281, 118804. doi:10.1016/j.fuel.2020.118804.

Contribution to the papers included in the thesis

Manuscript I. In this research, I was responsible for the design, start-up and operation of the experimental set-up in collaboration with Dr. Alma Toledo-Cervantes and under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz. I performed the results evaluation and the preparation of the manuscript in collaboration with Dr. Esther Posadas under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz.

Manuscript II. In this work, I was responsible for the design, start-up and operation of the experimental set-up in collaboration with AQUALIA FCC and under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz. I performed the results evaluation and the preparation of the manuscript under the supervision of Dr. Raquel Lebrero, Dr. Pedro A. García-Encina and Dr. Raúl Muñoz.

Manuscript III. In this research, I was responsible for the design, start-up and operation of the experimental set-up, results evaluation and preparation of the manuscript in collaboration with Dr. Andrea Carvajal and under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz. Víctor Castro and Dr. Andrea Carvajal were responsible for the control system implementation, where I collaborated under the supervision of Dr. Daniel Navia and Dr. César de Prada.

Manuscript IV. In this work, I was responsible for the design, start-up and operation of the experimental set-up under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz. I performed the results evaluation and the preparation of the manuscript under the supervision of Dr. Andrea Carvajal, Dr. Raquel Lebrero and Dr. Raúl Muñoz. Dr. César de Prada collaborated with the control strategy implementation.

Manuscript V. During this research, I was in charge of the design, start-up and operation of the experimental set-up, results evaluation and manuscript preparation in collaboration with Dr. Ramasamy Praveenkumar and under the supervision of Dr. Koenraad Muylaert, Dr. Raquel Lebrero and Dr. Raúl Muñoz. Miss An Verfaillie and Dr. Jonas Blockx were responsible for the preparation of flocculants and collaborated with the manuscript revision under the supervision of Dr. Wim Thielemans.

Manuscript VI. In this work, I was responsible for the design, start-up, operation of the experimental set-up, results evaluation and preparation of the manuscript in collaboration with Cristian Alfredo Severi and Ricardo Rocher-Rivas under the supervision of Dr. Guillermo Quijano and Dr. Raúl Muñoz.

Chapter 1

Introduction

1.1. The need for biogas upgrading, global market and future trends

Today, fossil fuels constitute the major source of energy generation at global scale. Oil, coal and gas accounted for 31.8, 27.1 and 22.2%, respectively, of the world total primary energy supply in 2017 [1]. However, the rapid growth of the world energy consumption (~1.6-fold increase between 1990 and 2017) due to the increase in human population and industrial activity is compromising the availability of fossil fuel resources. Moreover, global warming, which mainly results from greenhouse gas emissions to the atmosphere during the combustion of fossil fuels, is a worldwide concern that encourages the development and utilization of renewable energy sources [2]. In this context, biogas production from the anaerobic digestion of organic waste such as municipal organic waste, livestock manure or wastewater treatment sludge can partially reduce the current fossil fuels dependence and their associated greenhouse gas emissions with the added benefit of organic waste treatment [3]. As a result of feedstock availability and national policy support, the global biogas production reached 1.31 exajoule in 2016, equivalent to a total volume of biogas of 60.8 billion Nm^3 y^{-1}, of which 54% corresponded to Europe [4]. In Europe, the installed electric capacity of biogas plants was 11082 megawatt (MW) in 2018, with a total number of biogas plants of 18202, which represented an increase of 11% in the installed electric capacity with respect to 2016 [5].

Biogas is typically composed of methane (40–75%), carbon dioxide (15–60%) and lower concentrations of other components such as hydrogen sulfide (0.005–2 %), oxygen (0–1%), nitrogen (0–2%), ammonia (<1%), carbon monoxide (<0.6%), siloxanes (0–0.02%), halogenated hydrocarbons (VOC< 0.6%) and water (5–10%) [6]. Due to its high CH_4 content, biogas is commonly used directly as household fuel for cooking, or to produce heat and electricity at *on-site* co-generation or only-electricity generation facilities (which requires low H_2S or siloxane concentration) [7]. The high content of CO_2 increases carbon dioxide emissions during biogas combustion, reduces biogas calorific value and increases its transportation and compression costs, which limits the economic feasibility of biogas. In this context, the energy content of biogas (CH_4 concentration of 60%) expressed by the lower calorific value is ~21 MJ m^{-3}, while in natural gas this value averages 36 MJ m^{-3} [8]. On the other hand, other biogas components such as H_2S, NH_3 and halocarbons are toxic and/or generate corrosion in pipelines, storage tanks and internal combustion engines [6]. These biogas pollutants

must be removed (in a process called biogas upgrading) to enable biomethane use as fuel in natural gas-powered vehicles or its injection into natural gas grids, which requires concentrations in biogas of $CH_4 \geq 90\%$, $CO_2 \leq 2–4\%$, $O_2 \leq 1\%$ and H_2S + COS < 5 mg Nm^{-3} according to most international regulations (including the recent European Standard UNE-EN 16723).

The European Renewable Energy Directive (RED II) targets a 32% consumption of energy from renewable sources by 2030, including a contribution of 14% of renewable energy in the transport sector by 2030 and an annual increase of 1.3% in the share of renewable energy in the heating sector [9]. The low profitability of electricity biogas plants and the new opportunities for biomethane use in the transport sector has encouraged biogas upgrading in Europe during the last years. Indeed, Europe is nowadays the world's leading producer of biomethane [10]. For instance, the number of biomethane plants in Europe has increased from 187 in 2011 to 660 in 2018, with a biomethane production up to 22737 GWh in 2018 (Fig. 1). However, biogas upgrading is still marginal in most countries, with an estimated number of biomethane plants in non-European countries of 160 in 2017 [4].

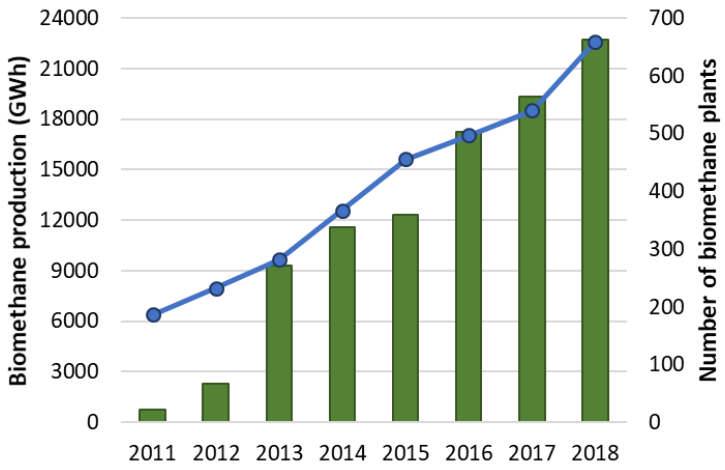

Fig. 1. Development of biomethane production in GWh (green bars) and number of biomethane plants (blue line) in Europe (EBA Database).

Biomethane is a promising energy carrier that could reach a production of 1072 TWh (22% of current natural gas consumption) in 2050 according to "Gas for Climate: a path to 2050" initiative [11]. Nevertheless, the development of a cost-competitive and environmentally friendly biogas upgrading technology is still necessary to boost the use of this energy source [10].

1.2. End-of-the-pipe technologies for biogas upgrading

1.2.1. Physical/chemical technologies

Currently, physical/chemical technologies are widely applied for biogas upgrading in the European market due to their high efficiency and commercial availability (Fig. 2). However, these technologies require high energy and chemical demands, which limits the use of biomethane as a green technology.

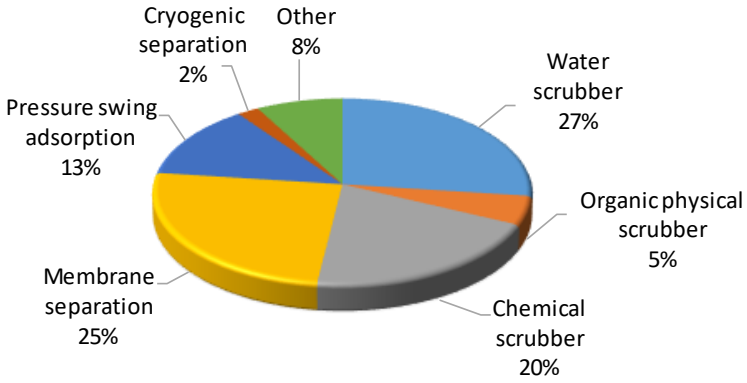

Fig. 2. Market share of CO_2 removal technologies in the European Union (EBA Database).

1.2.1.1. CO_2 removal

Physical and chemical absorption

CO_2 removal by absorption relies on the higher solubility of CO_2 in a scrubbing liquid solution (water, organic solvent or chemical solution) compared to CH_4. Water or organic solvent scrubbing is based only on the gas-liquid mass transfer of CO_2 (physical absorption) in a packed column operating at high pressures (6-10 bar) under counter-current mode. Treated water from wastewater treatment plants can be used in single-pass scrubbers, however, when tap water is supplied, a two-stage stripping process (a flash column to recover most of the dissolved methane followed by a CO_2 desorption column) after CO_2 absorption is recommended for water regeneration (Fig. 3a) [12]. The use of organic solvents, such as methanol or polyethylene glycol, allows for a reduction in plant sizing and liquid recycling rates due to their higher affinity for CO_2 than water [6]. However, organic scrubbing requires a biogas pretreatment and a solvent cooling to 20ºC prior CO_2 absorption and a heating stage to 40ºC for solvent regeneration (Fig. 3b) [13]. Despite biomethane quality of 95-99% and CH_4 losses lower than 2% can be achieved using physical absorption processes, this technology exhibits high energy costs [14].

Chemical scrubbing typically uses alkanol amines or alkali aqueous solutions (i.e. NaOH, KOH, CaOH) to react with the CO_2 absorbed in the liquid, forming HCO_3^-/CO_3^- species that boost CO_2 gas-liquid mass transfer. This process entails lower liquid recycling rates and operation at low pressure in the absorption column (1-2 bar) with CH_4 concentrations in the biomethane up to 99.8% and CH_4 losses of 0.1%. However, solvent regeneration requires temperatures of 120-150ºC, thus increasing the overall energy costs and requiring a H_2S pretreatment prior amine scrubbing (Fig. 3c).

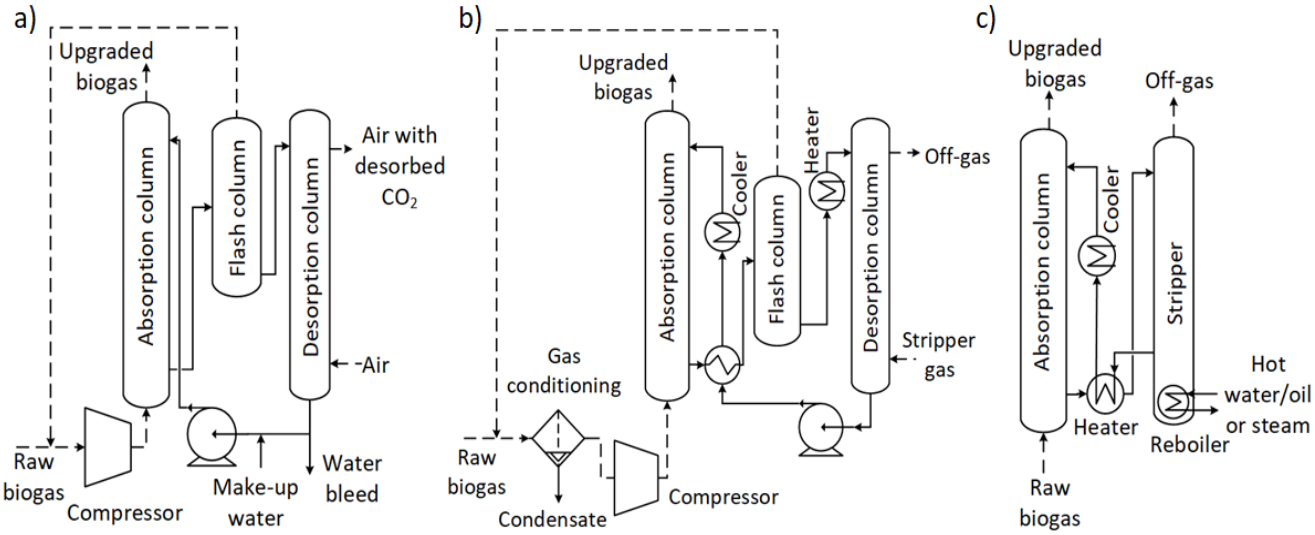

Fig. 3. CO_2 removal by absorption: a) water scrubbing, b) organic solvent scrubbing, c) chemical scrubbing. Adapted from Bauer et al. [13].

Pressure swing adsorption

Pressure swing adsorption (PSA) is based on the lower size of CO_2 molecules and its higher affinity to certain adsorbents in comparison with CH_4, which allows the selective retention of CO_2 by a solid phase while CH_4 molecules pass through the interstitial spaces of the adsorbent unit [15]. The adsorbents typically used are activated carbon, zeolite, activated alumina, silica gel or polymeric solvents with a high surface area [6,15]. Conventional PSA units consist of 4 interconnected vertical columns packed with the adsorbent working in a different stage: pressurization, adsorption at 4-10 bar to increase CO_2 retention, depressurization by venting and regeneration of the adsorbent by purging with the upgraded biogas (Fig. 4) [12]. Despite this process is capable of providing a CH_4 concentration in the upgraded biogas of 96-98% with CH_4 losses of 2-4%, PSA requires drying the biogas and removal of H_2S prior injection of the biogas in the PSA columns [16].

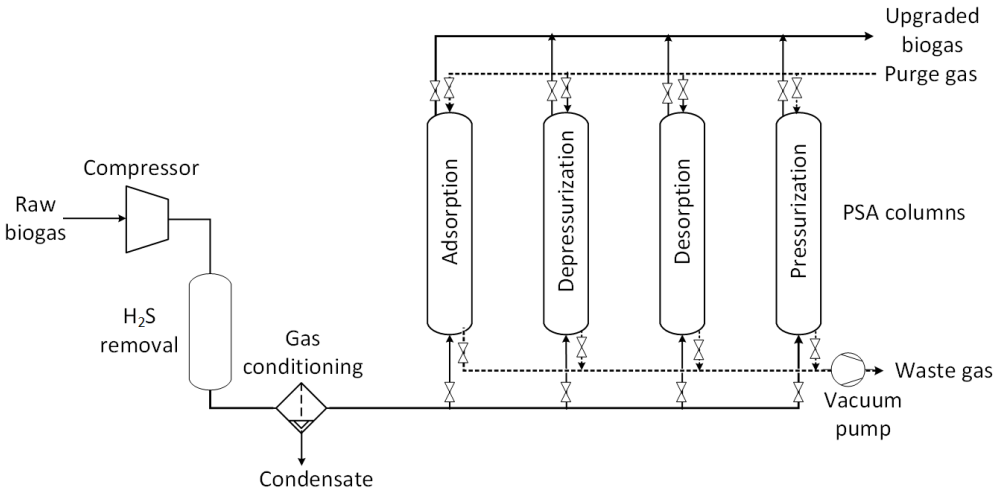

Fig. 4. CO_2 removal by pressure swing adsorption (PSA). Adapted from Bauer et al. [13].

Membrane separation

Membrane-based processes for CO_2 removal are based on the selective permeability of specific membranes, which allows CO_2 to pass through the membrane while CH_4 is retained. This technology operates at atmospheric pressure in gas-liquid modules using alkanol amines or alkali aqueous solutions on one side of the membrane, or at high pressure (20-40 bar) in gas-gas modules [12]. Gas-gas units need multiple membrane stages and internal recirculation of permeates and retentates to increase CH_4 recovery and avoid CH_4 losses (CH_4 concentrations of 10-25% are typically found in the permeates; Fig. 5) [6]. Although CH_4 concentrations between 96 and 98% can be reached in gas-liquid modules or multiple-stage gas-gas modules, [3,6], the main drawback of this technology is the high maintenance cost due to the need for a periodical membrane replacement [16].

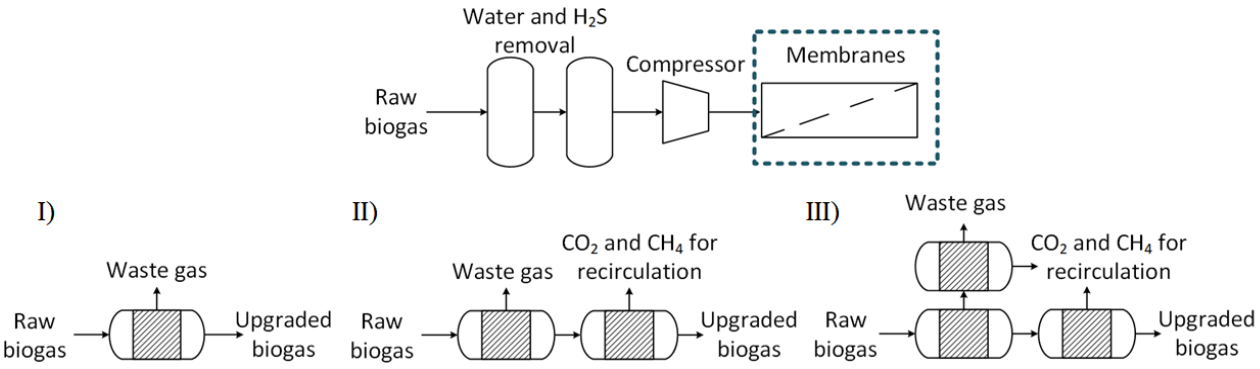

Fig. 5. CO_2 removal by membrane separation. Different configurations of gas-gas units: I) single-pass membrane, II) multiple stage membrane units with internal recirculation of permeate and III) internal recirculation of permeate and retentate. Adapted from Bauer et al. [13].

Cryogenic separation

This technology consists on the selective separation of biogas components based on their different liquefaction/solidification points. The process typically entails a sequential biogas compression till 80 bar followed by a stepwise temperature drop down to -45 and -55ºC to remove CO_2 via liquefaction, and a further expansion to 8-10 bar reaching a temperature of -110ºC, where CO_2 in solid phase is separated from the biomethane (Fig. 6) [6]. This process needs a previous step for the removal of water, H_2S, siloxanes and halocarbons to avoid freezing or clogging [12]. Despite a high purity biomethane (CH_4 > 97%) with limited CH_4 losses (< 2%) can be achieved, cryogenic separation still exhibits high investment and operation costs [3,13].

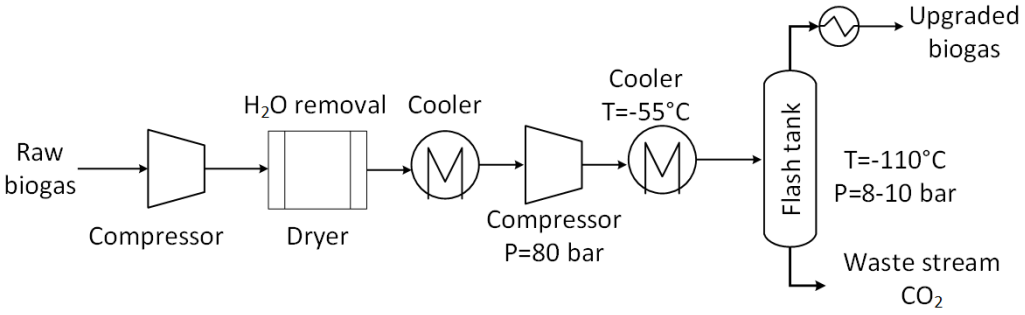

Fig. 6. CO_2 removal by cryogenic separation. Adapted from Adnam et al. [17].

1.2.1.2. H_2S removal

Adsorption using metal oxides or hydroxides

This technology is based on the chemical adsorption of H_2S on the surface of metal oxides or hydroxides such as Fe_2O_3, ZnO and $Fe(OH)_3$ supported onto wood chips or pellets made of red mud [8]. The unit operation usually consists of two parallel modules for H_2S removal (Eqs. 1 and 2) and the subsequent regeneration of the adsorbent material with air (Eq. 3) (Fig. 7a).

$$Fe_2O_3 + 3\ H_2S \rightarrow Fe_2S_3 + 3\ H_2O \quad (1)$$

$$2\ Fe(OH)_3 + 3\ H_2S \rightarrow Fe_2S_3 + 6\ H_2O \quad (2)$$

$$2\ Fe_2S_3 + 3\ O_2 \rightarrow 2\ Fe_2O_3 + 6\ S \quad (3)$$

Both reactions involved in H_2S oxidation are endothermic, while adsorbent regeneration is highly exothermic and might lead to auto-ignition if temperature is not rigorously controlled [6]. This process is simple and effective resulting in H_2S levels in

biomethane < 1 ppm_v [18]. However, the operating costs are high and it is recommended for biogas streams with H_2S concentrations up to 150 ppm_v [19].

Adsorption on activated carbon

H_2S can be removed by physical adsorption using non-impregnated activated carbon or by catalytic oxidation of H_2S to elemental sulfur, where activated carbon is impregnated with $NaHCO_3$, Na_2CO_3, NaOH, KOH, KI or $KMnO_4$. Adsorption via partial oxidation of H_2S (Eq. 4) requires the addition of 4-6% air to the biogas, temperatures of 50-70 ºC and pressures of 7-8 bar (Fig. 7b) [6].

$$2\ H_2S + O_2 \rightarrow 2\ S + 2\ H_2O \qquad (4)$$

In this method, carbon impregnated with KI or $KMnO_4$ is the most suitable option for biomethane use as vehicle fuel or natural gas substitute, since these compounds can support the partial oxidation of H_2S without air addition, thus avoiding O_2 content in the biomethane [18]. Regeneration with nitrogen or steam at high temperatures or replacement of the carbon is necessary after carbon saturation, which could entail between 4000 and 8000 h of operation subject to the H_2S loading rate [6,8].

In-situ H_2S precipitation

Addition of iron salts such as $FeSO_4$, $FeCl_2$ and $FeCl_3$ directly into the digester or to the organic influent effectively reduces H_2S concentrations in the biogas by reacting with the dissolved H_2S, leading to insoluble FeS and/or elemental sulfur formation (Eqs. 5 and 6) [6,12]:

$$Fe^{2+} + S^{2-} \rightarrow FeS \qquad (5)$$

$$2\ Fe^{3+} + 3\ S^{2-} \rightarrow 2\ FeS + S \qquad (6)$$

This process is simple and requires low investment costs (an iron salt storage tank and a dosing pump) (Fig. 7c). Nevertheless, the main drawbacks of this method are its limited efficiency to reduce H_2S levels below 100-150 ppm_v, accumulation of FeS in the digester, higher presence of iron in the effluent and high operating cost derived from the high cost of iron salts purchase [20,21].

Absorption

This process is based on the gas-liquid mass transfer of the H_2S using water or organic solvents (physical absorption) or via H_2S mass transfer prior to its conversion to metal

sulfides or elemental sulfur using aqueous chemical solutions (chemical absorption) [6]. Physical absorption can be implemented in a single pass or following by a solvent regenerative step, the latter being mandatory when using organic solvents due to their high costs. This method is only cost-effective in combination with CO_2 removal and suitable for the removal of low concentrations of H_2S (Fig.) [22]. On the other hand, chemical absorption using reagents such as NaOH, $Fe(OH)_3$, $FeCl_2$, Fe^{3+}/MgO, $Fe^{3+}/CuSO_4$ and Fe^{3+}/EDTA allows obtaining higher H_2S concentration gradients between the biogas and the solution resulting in lower liquid/biogas ratios [12]. In this context, the catalytic solution Fe^{3+}/EDTA is widely applied since the product of H_2S oxidation is elemental S, which can be easily removed by sedimentation followed by the regeneration of the solution by oxidation with O_2 (Fig. 7d). This technology achieves H_2S removals of 90-100% operating at ambient temperature and pressure and low biogas residence times [23].

Membrane separation

Similarly to CO_2 removal, H_2S can be separated from raw biogas by using certain membranes with a preferential permeation of H_2S and retention of CH_4 (Fig. 7e). High pressure gas-gas units or low pressure gas-liquid units using alkaline solutions are commercially available for H_2S removal. A complete H_2S removal has been reported in a gas-liquid membrane with NaOH solution as liquid absorbent at a pH of 10 operating at a gas retention time of 19 min [24]. Likewise, H_2S removal efficiencies of up to 94% were achieved with a polymeric membrane at feed flow rates of 25-41 kg/h and pressures between 4-8 barg [25]. This technology entails high operation costs and is not suitable for biogas streams with medium-high H_2S concentration.

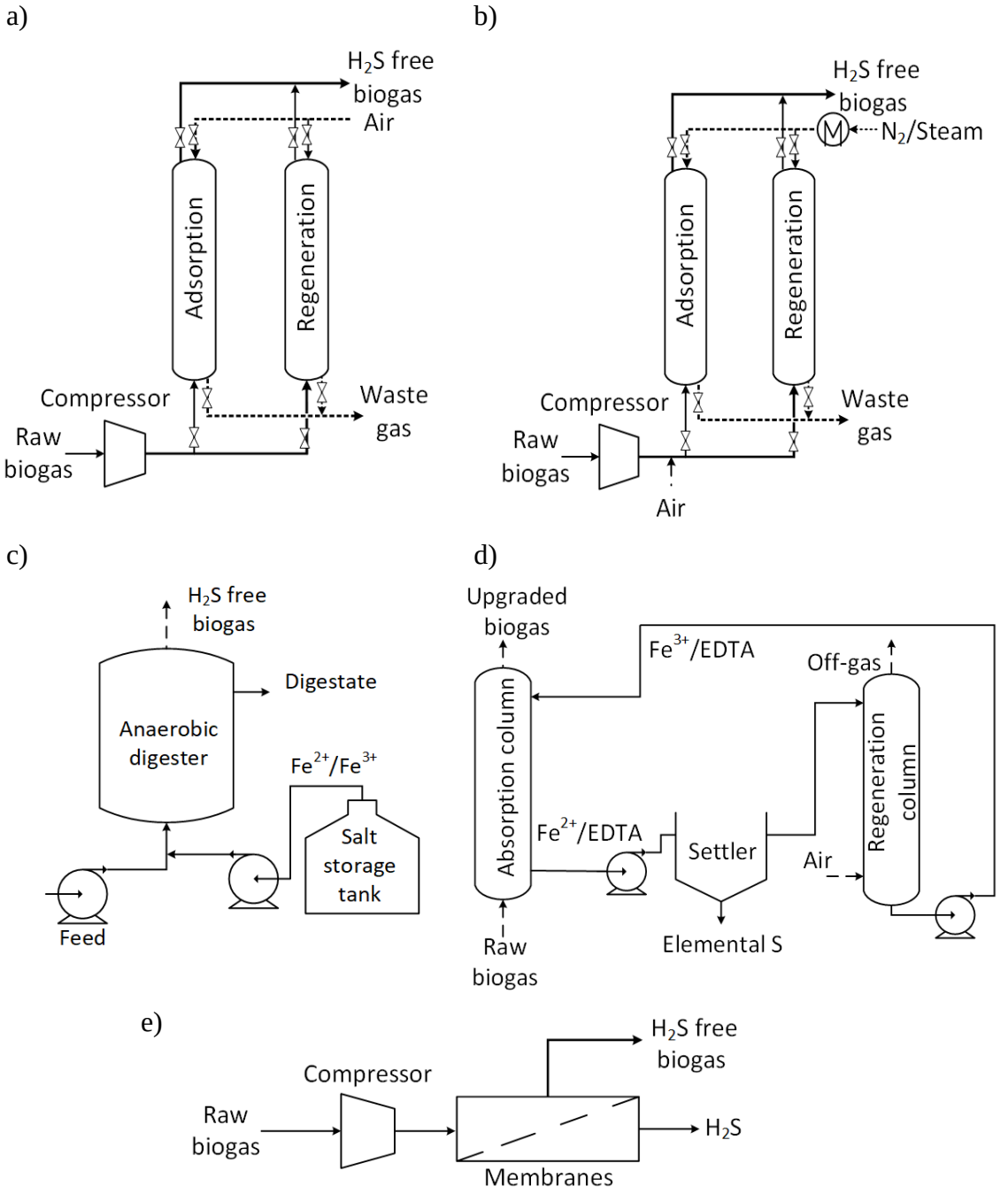

Fig. 7. Physical-chemical H_2S removal technologies: a) adsorption using metal oxides or hydroxides, b) adsorption on activated carbon, c) in-situ precipitation, d) chemical absorption and e) membrane separation.

1.2.2. Biological technologies

Biological technologies constitute a low-cost and environmentally friendly alternative to their physical-chemical counterparts. These technologies are currently being optimized at pilot scale and some of them (such as photosynthetic biogas upgrading) can support a simultaneous removal of CO_2 and H_2S.

1.2.2.1. CO_2 removal

Hydrogenotrophic CO_2 removal

Hydrogenotrophic biogas upgrading or biological methanation of CO_2 relies on the ability of hydrogenotrophic methanogens to convert the CO_2 present in biogas to CH_4 using H_2 as electron donor according to Eq. 7 [26]:

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O \qquad \Delta G^0 = -130.7\ kJ\ mol^{-1} \qquad (7)$$

The H_2 required for the bioconversion of CO_2 to CH_4 should come from a renewable origin in order to make this technology environmentally sustainable. In this context, surplus electricity from renewable sources such as solar panels or wind mills can be used for the production of H_2 via water hydrolysis prior biogas upgrading [27]. Moreover, H_2 could be also co-generated by dark fermentation processes [28].

Hydrogenotrophic methanogenic archaea play a key role during CO_2 conversion. Microorganisms from the genera *Methanobacterium*, *Methanoculleus*, *Methanomicrobium* and *Methanothermobacter* have been consistently found in bioreactors devoted to the conversion of CO_2 to CH_4 via H_2 injection [29–33]. These hydrogenotrophic methanogens often present an optimum activity at pH 6.5-8 under mesophilic and thermophilic condition [12]. In this context, methanogenic activity is not always the limiting step during hydrogenotrophic biogas upgrading, the low solubility of H_2 in water (dimensionless gas-water Henry's Law constant of 52 at 35ºC) typically limiting H_2 mass transfer from the gas to the aqueous phase that contains the methanogenic culture [34]. Hydrogen assisted CO_2 removal can be carried out in two different configurations: i) *in-situ* biogas upgrading, which involves supplying H_2 inside the anaerobic digester; and ii) *ex-situ* biogas upgrading, where biogas and H_2 are injected in an external bioreactor designed to maximize H_2 mass transfer and containing a hydrogenotrophic archaeal culture (Fig. 8) [35].

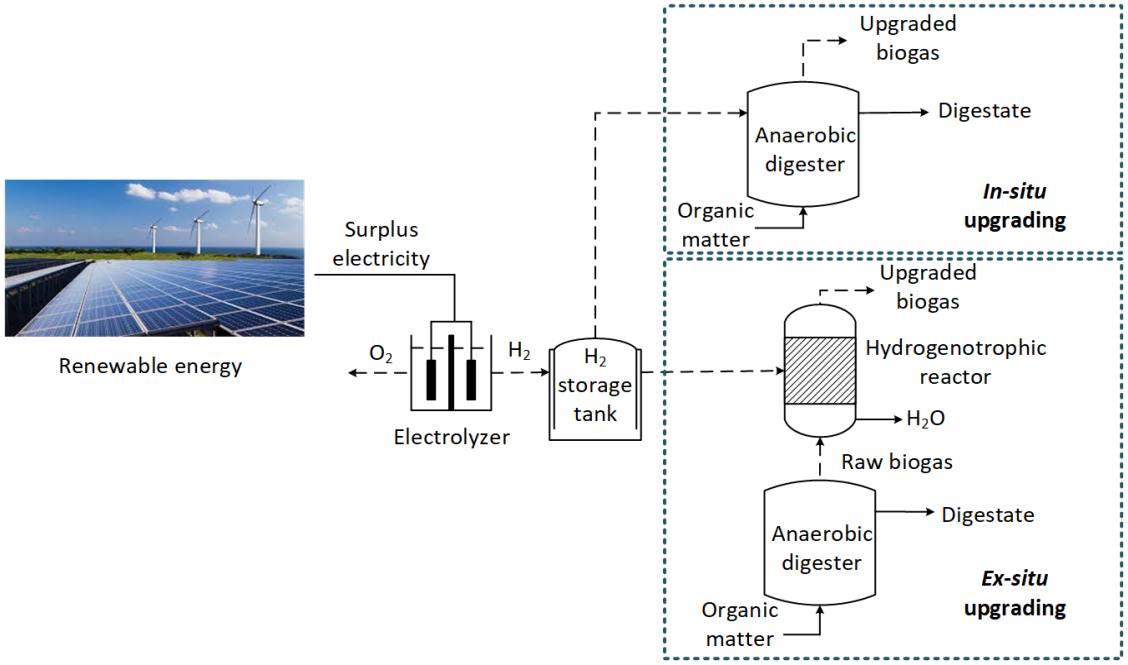

Fig. 8. Main configurations for the biological conversion of CO_2 into CH_4. Adapted from Rodero et al. [35].

In-situ biological biogas upgrading is limited by the low gas-liquid mass transfer since anaerobic digesters are designed to maximize the removal of organic matter but not H_2 absorption [36]. Another important operating issue in this process configuration is the increase in pH above 8.5 induced by the consumption of CO_2, which might result in methanogenesis inhibition [37]. On the other hand, *ex-situ* biomethanation requires a supplementary process unit for the upgrading of biogas, which represents an additional investment [38]. However, this process configuration does not affect organic matter degradation in the anaerobic digester, thus making the biochemical process simpler and more flexible (allowing the treatment of different CO_2 residual sources) [16]. Recently, a hybrid configuration, in which *in-situ* upgrading results in the conversion of part of the CO_2 present in biogas into CH_4 prior to the *ex-situ* process has been proposed. This integrated system can solve the problem of the pH increase during the *in-situ* process, while reducing the reactor volume needed in the *ex-situ* process [32]. However, further optimization of this process configuration is needed.

1.2.2.2. H_2S removal

Biotrickling filtration

Biotrickling filters (BTF) consist of packed bed columns (where biomass growth occurs as a biofilm) sprayed by a recirculating aqueous phase that contains the essential

nutrients for microbial growth. In aerobic BTF, lithoautotrophic bacteria can use H_2S as the energy source while O_2 is used as the electron acceptor according to Eqs. 8 and 9:

$$H_2S + 0.5\ O_2 \rightarrow S + H_2O \quad (8)$$

$$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2\ H^+ \quad (9)$$

The control of the oxygen dosage into the BTF is critical due to both safety concerns (explosion risks) and to the need to avoid biogas dilution (Fig. 9a) [39]. NO_3^- or NO_2^- can be also used in anoxic BTFs as electron acceptor for the biological oxidation of H_2S, which would contribute to a concomitant nitrogen removal from digestates via denitrification (Fig. 9b) [40]. The stoichiometry of H_2S removal via nitrate reduction is described by Eqs. 10 and 11 [39,41].

$$5\ H_2S + 2\ NO_3^- \rightarrow 5\ S + N_2 + 4\ H_2O + 2\ OH^- \quad (10)$$

$$5\ H_2S + 8\ NO_3\text{-} \rightarrow 5\ SO_4^{2-} + 4\ N_2 + 4\ H_2O + 2\ H^+ \quad (11)$$

Elemental sulfur might be preferred over sulfate formation in order to avoid trickling liquid acidification [42]. However, the accumulation of elemental sulfur under oxygen or nitrate limiting conditions increases the risk of BTF clogging [43]. In this context, O_2/H_2S ratios of 2-41 and NO_3^-/H_2S ratios of 0.25-1.6 are recommended for an efficient H_2S oxidation in aerobic and anoxic BTFs, respectively [12,40,44].

Sulfur oxidizing bacteria (SOB) such as *Thiothrix* sp., *Thiobacillus* sp., *Thiomonas* sp., *Acidithiobacillus* sp. and *Sulfurimonas* sp. are capable of oxidizing H_2S under neutral/basic pH conditions using the CO_2 present in biogas as a carbon source [45,46]. Process operation under acidic pH conditions does not entail a reduction in the H_2S removal capacity as a result of the development of acidophilic bacterial biofilms of *Acidithiobacillus thioxidants*, *Acidiphilium* sp. and *Thiobacillus ferrooxidans* able to grow at a pH of 2 - 4 [47,48]. High removal efficiencies of 80-100% have been achieved under anoxic and aerobic conditions with inlet H_2S concentrations in the range 500-10000 ppm_v [12]. However, elemental sulfur accumulation is nowadays considered the bottleneck limiting the applicability of BTFs. Indeed, packing material replacement (HD-Q-PAC, polyurethane foam, pall rings or polypropylene carriers) represents the main cost during the operation of this biotechnology (up to 44% of the total operation cost) [49].

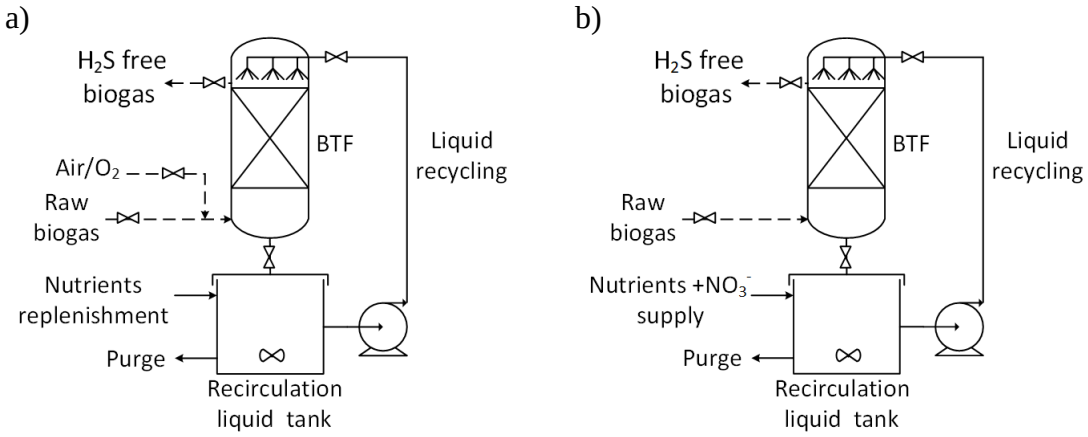

Fig. 9. H_2S removal by a) aerobic and b) anoxic biotrickling filter.

In-situ microaerobic H_2S removal

This biotechnology is based on the oxidation of H_2S to elemental sulfur via the action of SOBs under O_2-limited conditions in the headspace of the anaerobic digester according to Eq. 8. O_2 or air can be added directly to the headspace of the anaerobic digester or to the liquid phase with sludge recirculation, or even to the biogas when it is recirculated (Fig. 10). The O_2 supply rate is normally adjusted to 0.3-3% of the biogas production rate, although this parameter depends on biogas residence time and H_2S concentration [35,51]. SOBs from the genera *Acidithiobacillus*, *Arcobacter*, *Sulfuricuvum*, *Acinetobacter*, *Sulfurimonas*, *Thiobacillus*, *Thiofaba* and *Thiomonas* have been found at the headspace of microaerobic digesters [52,53]. This biotechnology avoids the use of an additional desulfurization unit with H_2S removal efficiencies > 97% at biogas residence time > 5 h [12]. Nevertheless, periodical cleaning of the digester headspace due to elemental sulfur deposition is the main factor governing a sustainable implementation of microaerobic conditions in full-scale reactors [54].

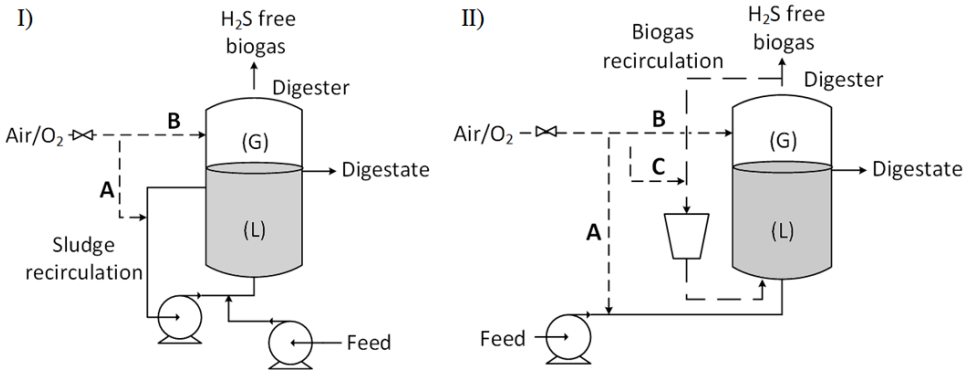

Fig. 10. Microaerobic digesters with I) sludge and II) biogas recirculation: **A** O_2/air dosage in the liquid phase, **B** dosage in the headspace of the digester, **C** dosage in the biogas recirculation. Adapted from Krayzelova et al. [55].

1.3. Photosynthetic biogas upgrading

1.3.1. Fundamentals

Biogas upgrading in algal–bacterial photobioreactors constitutes a promising alternative for the simultaneous removal of H_2S and CO_2 in a cost-effective and sustainable way [56]. Photosynthetic CO_2 removal is based on the biofixation of the CO_2 present in the biogas by eukaryotic microalgae and prokaryotic cyanobacteria (from now on referred to as microalgae) using solar radiation, which generates a valuable microalgae biomass. During this redox process, known as oxygenic photosynthesis, the electrons released during water photolysis are used to reduce the CO_2 present in biogas. In addition, this biotechnology can support the concomitant oxidation of H_2S to sulfate or elemental sulfur by aerobic SOB using the oxygen photosynthetically produced by microalgae (Fig. 11). These processes can be stoichiometrically described as follows (Eq. 12, 13) [12,48]:

$$CO_2 + H_2O + \text{photons} + \text{nutrients} \rightarrow O_2 + CH_{1.63}N_{0.14}O_{0.43}P_{0.006}S_{0.005} + \text{waste heat} \quad (12)$$

$$H_2S + CO_2 + \text{nutrients} + O_2 \rightarrow \text{bacterial biomass} + SO_4^{2-}/S + H_2O \quad (13)$$

The addition of nutrients (N, P and other trace elements) in the cultivation broth is mandatory to support microalgal-bacterial growth and the subsequent CO_2 sequestration and H_2S oxidation [57]. In this context, domestic wastewaters and anaerobic effluents have emerged as an inexpensive nutrient and water source that ultimately reduce the associated operational costs of this technology [58]. Moreover, microalgal-bacterial biomass is obtained as a byproduct, whose productivity will depend mainly on the set-up configuration, nutrient availability and environmental conditions. In this context, approximately 1.8 g of CO_2 are needed per gram of microalgae generated [59]. Since microalgal biomass is composed of 40-60% carbon, 4-9% nitrogen and 0.2-3.9% phosphorous [60,61], these nutrients can be further recycled by using microalgal biomass as biofertilizer or feedstock for biofuel production (i.e. biogas), thus increasing the sustainability of this process.

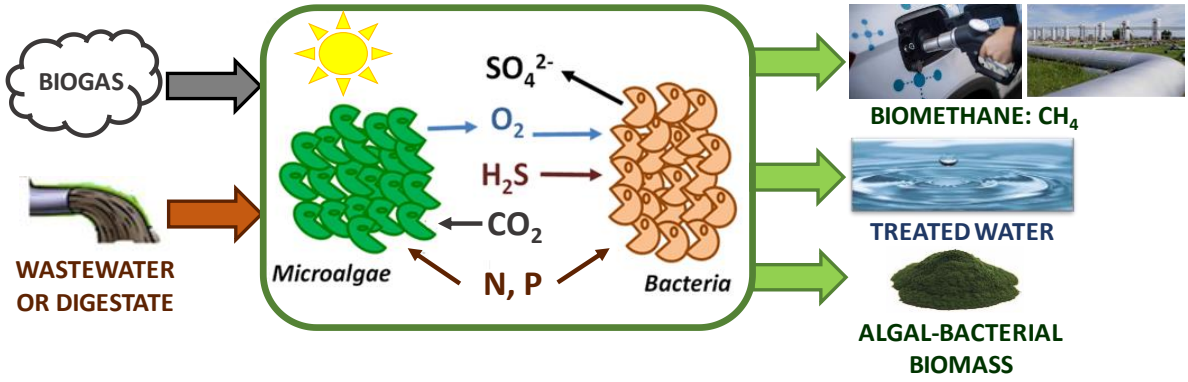

Fig. 11. Algal-bacterial symbiosis during photosynthetic biogas upgrading.

Photosynthetic biogas upgrading requires a previous CO_2 and H_2S mass transfer from the raw biogas to the aqueous cultivation broth, the limiting step being CO_2 removal due to the three times higher H_2S aqueous solubility according to their Henry's Law constants (dimensionless water-gas Henry's Law constant of 0.83 and 2.44 at 25 °C for CO_2 and H_2S, respectively) and to the rapid biological H_2S oxidation [12,34]. In this regard, process operation at high pH values (9-10) in the cultivation broth enhances CO_2 and H_2S mass transfer (as explained in section 1.3.2). Some microalgae such as *Chlorella*, *Scenedesmus*, *Anabaena* and *Spirulina* can support photosynthetic biogas upgrading due to their tolerance to a wide range of pH and high CO_2 concentrations [62]. Indeed, no inhibitory effect on isolated microalgae have been found at CO_2 concentrations of up to 40-60% [63,64]. Moreover, CH_4 has a poor aqueous solubility, which prevents any microalgae growth inhibition while minimizing CH_4 losses ($\leq$ 5%) during the process of biogas upgrading [65,66]. Finally, the rapid H_2S oxidation to sulfate mediated by alkaliphilic SOB and the high dissolved oxygen concentrations prevailing in the cultivation broth, prevents H_2S inhibition on microalgae activity (H_2S concentration $\geq$ 100 ppm_v) [65,67]. Actually, a recent study has identified bacteria from the genus *Thioalbus* in the algal-bacterial broth, which supported the biological nature of H_2S oxidation in photobioreactors devoted to biogas upgrading [66].

On the other hand, an appropriate design and operation of the photobioreactor is necessary in order to improve CO_2 removal from biogas and microalgae growth [34]. High rate algal ponds (HRAPs) or closed photobioreactors such as bubble column and horizontal tubular photobioreactors are the most common configurations used for biogas upgrading. HRAPs require lower capital investment and operation costs than their closed counterparts, and thereby are considered the best configuration for low-cost algal biomass production [68]. However, HRAPs typically present low biomass

concentrations in the cultivation broth (0.3–1.2 g total suspended solid (TSS) L^{-1}) and low biomass productivities (5–30 g m^{-2} d^{-1}) due to their low photosynthetic efficiency (~2%) [69–71]. Consequently, HRAPs entail large land requirements for biogas upgrading and a high water footprint (up to 9 L m^{-2} d^{-1} during summer in temperate climates) [72,73]. In contrast, closed photobioreactors need high investment costs and energy requirements, but can support biomass concentrations of 2-8 g TSS L^{-1} and biomass productivities of 25–45 g m^{-2} d^{-1} as a result of their higher light utilization efficiency (4-6%) due to the higher turbulence and illuminated surface-volume ratio [69,74]. Biogas can be introduced either directly via biogas sparging in the photobioreactor or in an external absorption column where the CO_2-containing cultivation broth is recirculated to the photobioreactor. The former configuration entails a poor CO_2 removal in HRAPs due to low gas-liquid contact times, resulting in a low CO_2 gas-liquid mass transfer [68]. On the other hand, the main constraint of enclosed photobioreactors is the build-up of oxygen concentrations produced as a result of the high algal photosynthetic activity, which could lead to a high oxygen concentration in the upgraded biogas and produce explosive mixtures of CH_4/O_2 [75,76].

In this context, the engineering of an external bubble column interconnected to the photobioreactor improves the gas-liquid mass transfer and promotes lower oxygen content in the upgraded biogas than the single stage process (Fig. 12) [80]. Therefore, HRAP interconnected to an external bubble column represents an efficient and cost-competitive configuration for the simultaneous biological removal of CO_2 and H_2S from biogas [68]. In addition, the absence of packing material in the biogas scrubbing unit together with the high O_2 concentration prevailing in the algal-bacterial cultivation broth during biogas upgrading, prevent the clogging problems typically encountered in biotrickling filters due to elemental sulfur accumulation [39]. H_2S removal efficiencies of 100 % concomitant with CO_2 removals of 70-95% are typically reported during photosynthetic biogas upgrading in HRAPs interconnected to an absorption column at lab scale (Table 1). Despite these promising results, the validation of this biotechnology at semi-industrial scale is a requirement prior its full-scale implementation.

Table 1. Experimental studies on photosynthetic biogas upgrading under different configurations

Photobioreactor and absorption unit design	**Gas residence time (h)**	**L/G ratio**	**CO_2-RE (%)**	**H_2S-RE (%)**	**CH_4 (%)**	**O_2 (%)**	**pH**	**Microalgae population**	**References**
Indoors closed photobioreactor of 1 L	96	-	100	-	70-76	10-24	9.5	*Spirulina platensis*	[77]
Outdoors closed photobioreactor of 1 L	-	-	98	100	50-53	18-23	5.5-7.0	*Chlorella vulgaris*	[76]
Outdoors set of 50 L bubble columns	0.06-0.3	-	74-86	-	86-91	-	-	Mutant *Chlorella* sp. strain (MB-9)	[65]
Indoors 180 L HRAP interconnected to a 0.8 L bubble column	0.7	0.4-1.6	40-95	100	-	0.2-1.0	7-10	*Spirulina platensis*, *Phormidium*, *Oocystis*, *Microspora* sp.	[56]
Indoors 180 L HRAP interconnected to a 2.5 L bubble column	1.4-8.3	0.5-67	80	100	-	0.3-3	8	*Chlorella* sp., *Pseudanabaena* sp., *Chloromonas* sp., *Geitlerinema* sp., *Microspora* sp., *Stigeoclonium* sp. and *Planktolyngbya* sp.	[78]
Indoors 180 L HRAP interconnected to a 2.5 L bubble column	1.4	10.7	72-79	100	81	0.7-1.2	8	*Geitlerinema* sp., *Limnothrix planktonica*, *Pseudanabaena minima*, *Stigeoclonium tenue*, *Leptolyngbya benthonica*, *Planktolyngvya brevicellularis*, *Staurosira* sp.	[79]
Indoors 75 L HRAP interconnected to a 0.7 L bubble column	0.1-1.2	-	93	-	-	5	-	*Nannochloropsis gaditana*	[80]
Indoors 180 L HRAP interconnected to a 2.5 L bubble column	1	0.3-1.0	97-99	97	95-96	0.7-1.2	10.2	*Chlorella minutissima*	[66]

Photobioreactor and absorption unit design	**Gas residence time (h)**	**L/G ratio**	**CO_2-RE (%)**	**H_2S-RE (%)**	**CH_4 (%)**	**O_2 (%)**	**pH**	**Microalgae population**	**References**
Indoors 25 L HRAP interconnected to a 0.35 L bubble column	0.4	5.0	89-94	99-100	-	-	9.3-9.7	*Picochlorumsp*. and *Halospirulina* sp.	[81]
Outdoors 180 L HRAP interconnected to a 2.5 L bubble column	0.8	0.5	50-95	100	72-93	0.1-2.0	9-10	*Chlorella* sp., *Chloroidium saccharophilum* and *Pseudanabaena* sp.	[73]
Outdoors 180 L HRAP interconnected to a 2.5 L bubble column	0.8	1.0	64-96	100	85-98	0-3.4	9.2-9.8	*Chlorella vulgaris*, *Pseudanabaena* sp., *Chlorella kessieri* and *Leptolyngbya lagerheimii*	[82]
Indoors 60 L closed photobioreactor interconnected to a 3.5 L bubble column	1.5	1-11	57-100	97	83	8.3-9.6	7.2-10.7	*Acutodesmus obliquus*	[83]
Outdoors 11.7 m^3 semi-closed photobioreactor interconnected to a 45 L bubble column	10.8	0.5-5.0	>91	100	94-99	-	8-9	*Chlorella vulgaris*, *Stigeoclonium tenue*, *Nitzschia closterium*, and *Navicula amphora*	[84]

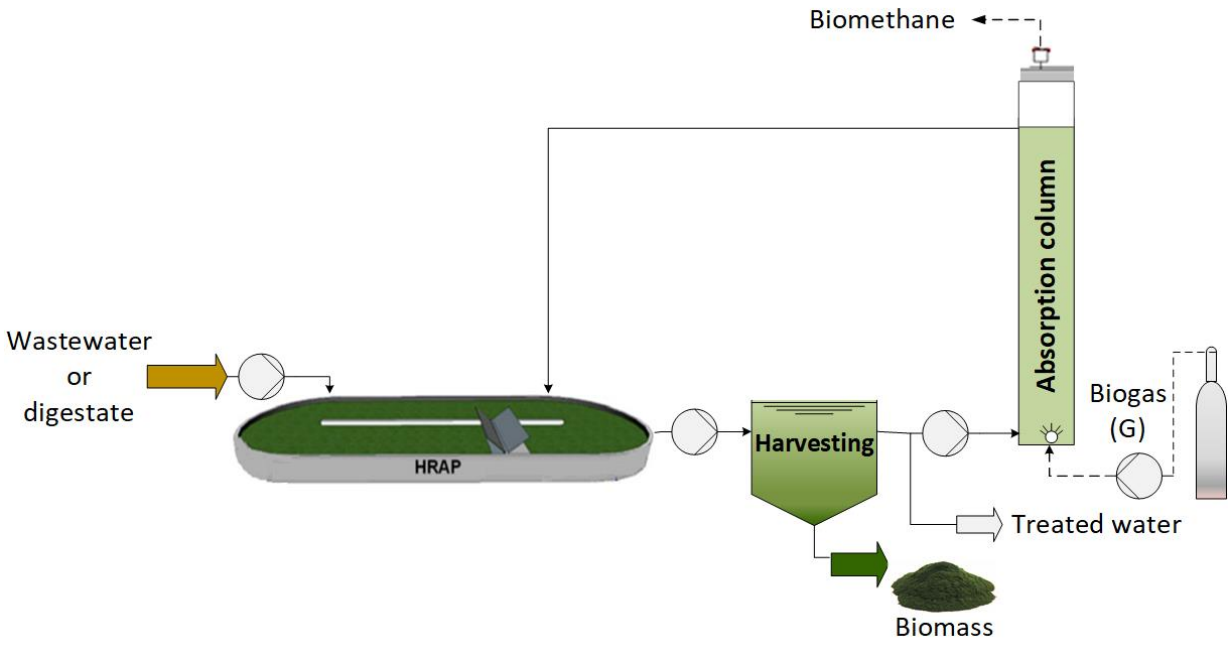

Fig. 12. Schematic diagram of the experimental set-up used in this thesis for the simultaneous biogas upgrading and wastewater treatment.

1.3.2. Parameters affecting photosynthetic biogas upgrading

Environmental conditions such as light availability, temperature and, indirectly, the dissolved oxygen (DO) concentration in the cultivation broth, impact on both CO_2 fixation by microalgae and the final quality of the upgraded biogas. In addition, the composition of the wastewater added as a nutrient and water source (section 1.3.3) is one of the main factors governing microalgae productivity and influencing key parameters in this process like the pH and the alkalinity of the cultivation broth. Furthermore, the optimization of the operational parameters in the system is a must to achieve a standard biomethane quality while improving microalgae productivity.

Light intensity and photoperiod

Light availability is a relevant factor affecting the rate and efficiency of the photosynthetic process since light provides the energy required to convert dissolved inorganic carbon into organic biomass via photosynthesis [85]. CO_2 capture rate increases with light intensity until it reaches a maximum where the culture becomes light saturated and microalgae growth remains constant. Higher intensities above the light saturation point can lead to photoinhibition or photodamage [86]. Most microalgae reach this saturation point at light intensities of ~200 μmol m^{-2} s^{-1}, which is approximately 8% of the summer and 17% of the winter maximum light irradiances in

temperate latitudes (2500 and 1200 μmol m^{-2} s^{-1}, respectively) [87]. However, due to the fact that about 10–20% of the total solar radiation is lost by reflection in the HRAPs and only 48% of the solar irradiance is photosynthetically active radiation (PAR; wavelength range from 400 to 750 nm), the maximum solar energy fixed by microalgae ranges from 1.3-2.4% depending on the climate, algal strains and operation conditions in the photobioreactor [70].

Microalgae growth is also affected by the length of the light/dark cycle since a long exposure to high irradiances may cause photoinhibition leading to decreased photosynthesis, while the dark period enables the recovery of the electron transport chain [88]. On the contrary, Jacob-Lopes et al. [89] reported a decrease in the biomass concentration and CO_2 fixation potential when the duration of the light period was reduced in *Aphanothece microscopica Nägeli* cultures at light intensity of 150 μmol m^{-2} s^{-1} as a result of a severe light limitation. Otherwise, Meier et al. [90] reported higher CO_2 removal efficiencies in the absorption column during the dark period, which was attributed to the temperature decrease in the absence of light. In contrast, a higher CO_2 stripping to the atmosphere was obtained during the dark period, which could eventually jeopardize the environmental sustainability of this biotechnology.

Temperature

Temperature governs most metabolic processes, which ultimately impacts on photosynthetic activity [91]. The optimal temperature for microalgae growth often ranges between 15 and 30°C, but it is highly species-specific, some strains being able to tolerate or even prefer lower or higher temperatures [92]. For instance, *Chlorella* sp. exhibits an optimal activity between 30 and 35°C [12]. A significant decrease in the metabolic activity of *Spirulina maxima* and *S. platensis* was recorded at temperatures below 17°C, while growth was not inhibited at 40°C [93]. On the other hand, low temperatures (2°C) positively impacted *Asterionella formosa* growth, being unable to survive at 27°C [94]. On the other hand, the solubility of the gases (CO_2, H_2S, O_2) increases when the temperature decreases, low temperatures thus supporting higher CO_2 and H_2S removal efficiencies in the absorption column [90]. In addition, other properties such as the ionic equilibria of the cultivation broth, the water evaporation rates and pH also depend on the temperature [95].

pH of the cultivation broth

During biogas upgrading coupled with wastewater treatment, the pH of the cultivation broth depends on the rates of algal/bacterial respiration, nitrification, CO_2 and H_2S mass transfer, photosynthetic activity of microalgae, and on the alkalinity and ionic composition of the wastewater [70]. The pH of the cultivation broth modifies the enzymatic activity and energetics of the cells associated with microalgal growth [96]. pH tolerance and the optimal pH value for microalgal growth differ among species. Despite most microalgae show a maximum activity at pH 7–8, acidophilic microalgae such as *Chlamydomonas acidophila* present an optimal growth at pH below 6, whereas the optimum pH reported for *Spirulina platensis* (alkaliphilic microalgae) is 10 [12,97,98]. Moreover, pH influences the NH_3/NH_4^+ equilibria and also phosphorus and heavy metals availability. Therefore, nutrient removal via NH_3 volatilization and orthophosphate precipitation occur at pH between 9 and 11 [99]. In addition, the pH of the cultivation broth impacts on the mass transfer phenomena associated to CO_2 and H_2S absorption from biogas and the distribution of their species in the liquid phase (Eqs. 14-17, pKa values at 25°C) [100]:

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \qquad pKa_1 = 6.35 \qquad (14)$$

$$HCO_3^- \leftrightarrow CO_3^{-2} + H^+ \qquad pKa_2 = 10.33 \qquad (15)$$

$$H_2S_{(aq)} \leftrightarrow HS^- + H^+ \qquad pKa_1 = 6.97 \qquad (16)$$

$$HS^- \leftrightarrow S^{2-} + H^+ \qquad pKa_2 = 12.90 \qquad (17)$$

In this context, a high pH in the cultivation broth increases the CO_2 and H_2S gas–liquid concentration gradient due to the acidic nature of these gases and consequently, their mass transfer in the absorption column [56]. Despite CO_2 consumption via photosynthesis increases the pH, photobioreactors with a high nitrification activity, which releases H^+ from NH_4^+ oxidation, and/or a continuous overload of biogas may undergo a severe acidification. In this context, low alkalinity systems might need alkali addition in order to compensate this acidification [101].

Alkalinity

The alkalinity in the cultivation broth plays a key role on CO_2 and H_2S mass transfer in the absorption column. A high alkalinity in the cultivation broth (high concentration of inorganic carbon) results in a high buffer capacity, which can sustain a limited decrease

of the pH along the absorption column [101]. Nevertheless, a high alkalinity inherently involves a high salinity in the photobioreactor cultivation broth, which might negatively impact on photosynthetic activity due to oxidative and osmotic stress on microalgae [102]. Moreover, high inorganic carbon concentrations tend to increase CO_2 stripping to the atmosphere, thus jeopardizing the environmental benefits of photosynthetic biogas upgrading.

Dissolved oxygen concentration

The large amounts of oxygen produced during the photosynthetic process (1.5 g O_2 per g of microalgae produced using NH_4^+ as a N source) might result in DO concentrations in the cultivation broth up to 40 mg O_2 L^{-1} [103]. High concentrations of DO (>25 mg L^{-1}) can inhibit the activity of some enzymes involved in photosynthesis (e.g., RuBisCO), induce light energy dissipation by photorespiration, or cause photochemical damages to membrane structures and to the photosynthetic apparatus, among others, which in turn results in a decrease in microalgal growth [104,105]. Moreover, high DO levels in the cultivation broth could result in a high O_2 desorption from the scrubbing liquid to the biogas and consequently, the production of an upgraded biogas unsuitable for use as a natural gas substitute or even explosion hazards [79]. In this context, the biological O_2 demand mediated by the oxidation of H_2S from biogas and organic matter or NH_4^+ from digestate or wastewater (which requires a minimum DO concentration of 2 mg L^{-1} to support the aerobic bacterial activity), partially mitigates this issue in algal-bacterial photobioreactors devoted to biogas upgrading [106].

Operational parameters in the absorption column

The **liquid to biogas (L/G) ratio** in the absorption column is a key operating parameter that must be optimized in order to achieve a high CO_2 and H_2S removal with a low O_2 and N_2 desorption from the liquid to the upgraded biogas. High L/G ratios entail an increase in the gas-liquid concentration gradients due to the lower acidification of the liquid along the absorption column, thus increasing the CO_2 and H_2S removal efficiencies. Nevertheless, an increase in the L/G ratio also enhances O_2 and N_2 stripping from the liquid to the upgraded biogas [78]. In this context, the optimum L/G ratio not only depends on the characteristics of the absorption unit (dimensions, configuration, diffuser type) but also on the environmental conditions and the type of wastewater used to support algal-bacterial growth, which directly influences the characteristics of the cultivation broth (i.e. pH, alkalinity, temperature). Hence, the L/G

ratio should be controlled over time in outdoors systems in order to guarantee an upgraded biogas complying with biomethane standards.

The **gas-liquid flow configuration** in the absorption column influences the biomethane quality. Counter-current flow operation is preferred in absorption units since it involves higher overall concentration gradients and mass transfer rates. Nonetheless, the superior O_2 and N_2 stripping along with operational problems such as elemental sulfur accumulation in the biogas diffuser due to the depletion of oxygen at the bottom of the column during counter-current configuration, counterbalance its beneficial CO_2 mass transfer rates. Therefore, co-current flow operation has provided the best performance during photosynthetic biogas upgrading [107]. Besides, the **biogas flowrate** should be optimized in order to improve the removal efficiency in the absorption column without exceeding the photosynthetic capacity of the photobioreactor. In this regard, an increase in the biogas flowrate maintaining a constant L/G could improve the gas-liquid mass transfer coefficient in the absorption column due to the higher turbulence induced.

Operational parameters in the photobioreactor

The **hydraulic retention time (HRT)**, defined as the volume of the photobioreactor divided by the inlet wastewater flowrate, determines the amount of nutrients supplied to the system and consequently, the biomass productivity under no light or CO_2 limitation [106,108]. The HRT must be optimized depending on the wastewater composition, environmental conditions and photobioreactor configuration in order to prevent biomass wash-out at low HRTs (which would entail a decrease in pH due to the lower photosynthetic activity) or nutrient limitation at high HRTs [109]. In this context, HRTs between 3 and 9 days are typically reported in HRAPs using domestic wastewaters while higher HRTs (>50 days) are commonly required when digestates or high-strength wastewaters are supplied due to their high nutrient content, which could lead to microalgae growth inhibition [66,110,111]. **Mixing** provides homogeneous conditions in the photobioreactor, limits the formation of anaerobic zones and supports the light/dark cycles that prevent photoinhibition [99]. Mixing must be optimized since it is energy-demanding, can generate shear stress in the microalgal-bacterial population and impacts on the gas/liquid mass transfer and, consequently, on the water evaporation rates and CO_2/NH_3 stripping in the photobioreactor [112]. In this regard, cultivation broth velocities of 15-30 cm s^{-1} are commonly applied in HRAPs [113].

1.3.3. Wastewater as a nutrient source

Wastewaters are characterized by their high content in carbon (organic and inorganic) and nutrients (mainly nitrogen and phosphorous) [114]. The concentration of these pollutants must be reduced before wastewaters are discharged into natural water bodies in order to avoid eutrophication, oxygen depletion and toxicity issues [115]. In this context, microalgae, which present a high tolerance to harsh environmental conditions, are able to grow in different types of wastewater and support a high nutrient removal and a cost-effective oxygenation potential [58]. Moreover, these effluents typically present a pH of 7–9, which matches the optimal range for microalgae growth [116]. In **domestic wastewaters**, most of the nitrogen is present as ammonium (NH_4^+), with low concentrations of nitrite and nitrate (Table 2). This feature favors nitrogen consumption by microalgae since NH_4^+ assimilation requires less energy than NO_3^- and NO_2^- conversion into structural nitrogen [117]. Despite domestic wastewaters present a C:N ratio (3.5:1) and a C:P ratio (20:1) too low in comparison with the optimum ratios for microalgae growth (C:N:P of 100:18:2), the CO_2 transferred from the biogas can compensate this C deficit [118,119].

Although domestic wastewaters can be used as a nutrient source, **digestates** (by-product of the anaerobic digestion) are preferred in photosynthetic biogas upgrading systems due to their higher pH (8-10) and alkalinity, which support a more cost-effective CO_2 and H_2S mass transfer to the aqueous phase (Table 2). In addition, nitrogen and phosphorous concentrations in digestates are considerably higher than in typical domestic wastewaters, although their composition varies depending on the type of organic waste digested, operational temperature, supplementation of trace elements, organic loading rates and the digester configuration [120]. On the other hand, **agro-industrial wastewaters** such as piggery wastewaters, which contain higher concentrations of organic matter, nitrogen, and phosphorus than domestic wastewaters, can be also used as nutrient source in microalgal-bacterial processes (Table 2) [121]. Similar to urban wastewater, the C:N:P ratios in digestates and agro-industrial wastewaters are lower than those required for microalgal growth and nutrient removal by assimilation. Otherwise, despite NH_4^+ is the preferred form of nitrogen for microalgae and bacterial growth, NH_4^+ concentrations >100 mg L^{-1} at pH > 8 decrease microalgae growth in some species due to the occurrence of free ammonia toxicity [119]. As a result, agro-industrial wastewaters and digestates must be diluted or fed at

low loading rates to microalgae-based treatment technologies (Table 3) [78,101]. In fact, these low feeding rates when using high-strength digestates along with high evaporation rates have resulted in a zero-effluent process operation [73,111]. Moreover, dilution strategies or pretreatment steps (e.g. oxidation via H_2O_2-UV combination or ozonation, the use of positively charged flocculants, biopolymers or adsorbents) contribute to reducing or removing the dark color of these effluents, thus avoiding problems of light limitation in the cultivation broth of the photobioreactors [122].

Table 2. pH and composition of different wastewaters

Parameters	**Domestic wastewater**	**Centrate**	**Piggery wastewater**
pH	7.1-7.8	8.3-9.2	7.3-7.6
COD (mg L^{-1})	395-1179	134-1043	987-11241
TOC (mg L^{-1})	112-292	16-891	3935-10340
IC (mg L^{-1})	68-186	450-974	1450-1750
TN (mg L^{-1})	49-166	316-1570	475-3680
N-NH_4^+ (mg L^{-1})	41-102	316-1143	364-655
N-NO_3^-(mg L^{-1})	0-0.5	0.2-8	<5
N-NO_2^-(mg L^{-1})	0-0.5	0	<5
TP (mg L^{-1})	10-52	45-297	44-85
P-PO_4^{3-} (mg L^{-1})	4-41	26-135	-
References	[58,114,123–125]	[111,126–128]	[129–131]

Finally, other pollutants such as heavy metals (Cu, Pb, Cd, Cr, or Zn) are commonly found in these wastewaters. Heavy metals can inhibit photosynthetic activity and bacterial growth even at low concentrations. For instance, Hamed et al. [132] reported the inhibition of *Chlorella sorokiniana* and *Scenedesmus acuminatus* growth when exposed to Cu concentrations of 1.6 and 3.2 mg L^{-1}, respectively. In contrast, some metals at trace level concentrations may improve microalgae growth. Indeed, Zhang et al. [133] observed an increase in *Ostreococcus tauri* growth at arsenic concentrations of 0.75–2.25 mg L^{-1}, while Huang et al. [134] reported that Cd concentrations of ~4.5 mg L^{-1} stimulated *Chlorella vulgaris* growth.

Table 3 compiles the removal efficiencies obtained during the simultaneous biogas upgrading coupled with wastewater treatment using microalgae-based processes.

Table 3. Average removal efficiencies (RE) obtained during the treatment of wastewaters coupled with biogas upgrading in photobioreactors

Wastewater	HRT (days)	COD-RE (%)	TOC-RE (%)	TC-RE (%)	TN-RE (%)	TP-RE (%)	Ref.
Diluted anaerobically digested vinasse	7.4	31-51	24-57	50-73	21-37	36-86	[78]
Diluted anaerobically digested vinasse / Raw vinasse	7.4	36-88	41-85	51-72	16-74	36-78	[79]
Synthetic digestate	146	-	-	72-87	91-98	63-77	[66]
Diluted digestate	7	-	-	20-44	40-100	45-82	[135]
Digestate	10	61-70	-	-	60-69	56-64	[136]
Digestate	180	81-93	-	-	97-99	90-99	[138]
Digestate	146	-	59-74	-	80-87	84-92	[101]

1.3.4. Microalgal-bacterial biomass harvesting

The separation of microalgal-bacterial biomass from the cultivation broth is a crucial step during photosynthetic biogas upgrading in order to use a biomass-free cultivation medium as scrubbing liquid to improve the gas-liquid mass transfer in the biogas absorption column, and to obtain a biomass that can be further valorized, thus increasing the economic feasibility of the process (as mentioned in section 1.3.1.). The low biomass concentrations typically encountered in HRAPs result in large volumes of cultivation broth to be managed to harvest a relevant biomass productivity. In this context, a suitable harvesting technology should be able to handle large volumes at a minimal cost and energy requirements [139]. The harvesting of algal biomass is affected by the species of microalgae, since they have different size, shape and cell wall composition, together with the composition of the cultivation broth such as algal organic matter or salt content [140]. Moreover, the harvesting process should not generate or introduce toxic substances that avoid microalgal-bacterial growth, since the clarified broth is recirculated to the photobioreactor during photosynthetic biogas upgrading, or that contaminate or alter microalgae biomass. Currently, harvesting methods such as gravity settling, flotation, centrifugation, filtration and flocculation, or a combination of them are typically applied for algal-bacterial biomass harvesting.

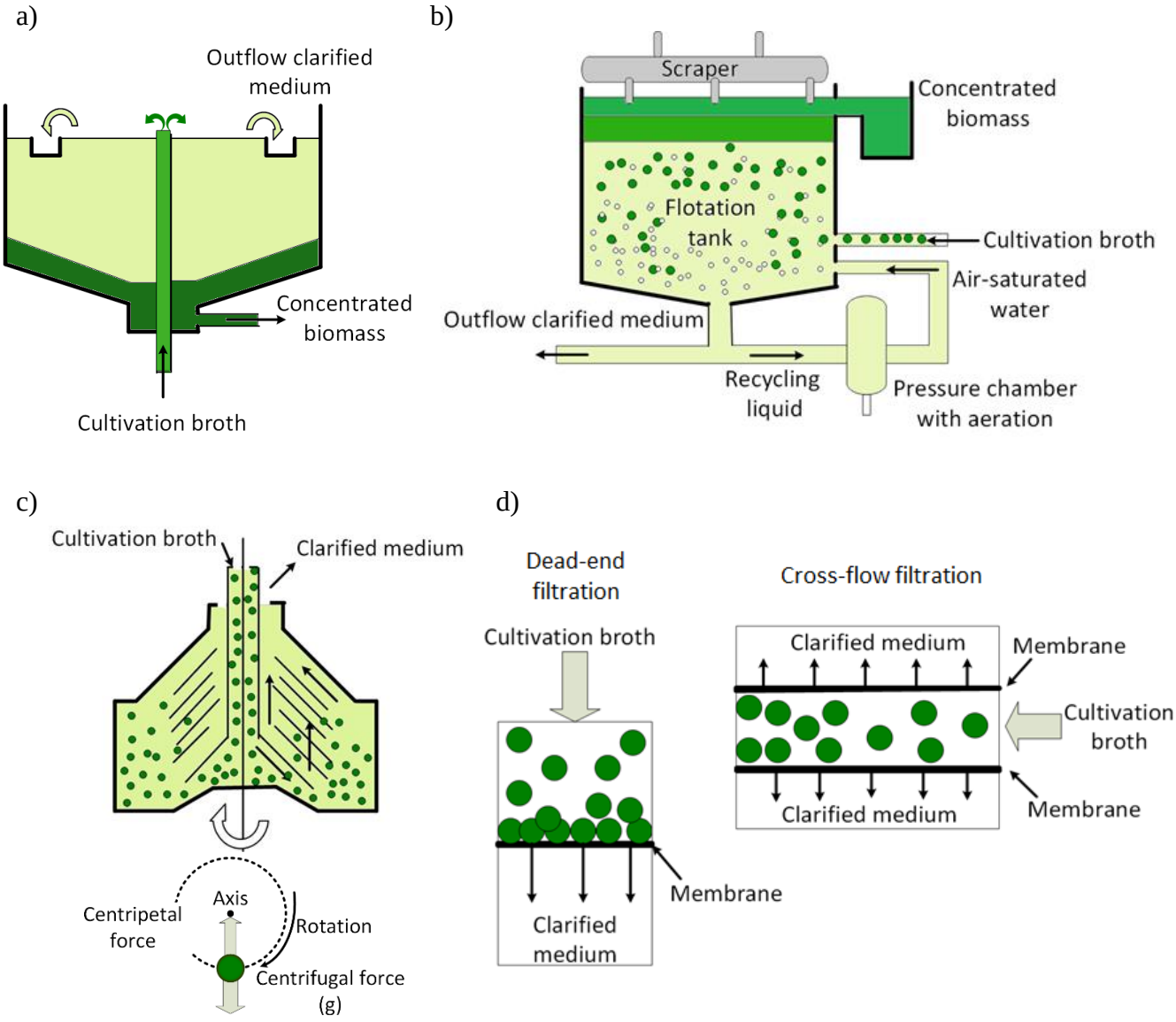

Fig. 13. Biomass separation by: a) gravity settling, b) dissolved air flotation, c) centrifugation, d) filtration. Adapted from Roselet et al. [141].

Gravity settling: This method is based on the separation of microalgae from the cultivation broth by the gravity force in settling tanks or lamella separators (Fig. 13a). Sedimentation is a simple process that requires low investment and operation costs. A key operational parameter in sedimentation is the settling rate of microalgae, which is determined by Stokes' Law. This law stablishes that the settling rate is proportional to the square of the radius of the cells (assuming spheroidal shapes) and the difference in density between the microalgae and the liquid [142]. Since most microalgae have a small size (<20 μm) and a density similar to that of water (1030-1140 kg m^{-3}), sedimentation is often very time-consuming (settling velocity ~1 cm h^{-1}), provides low cell recovery efficiencies (60-65%) and low biomass concentrations (<1.5% solids)

[143,144]. In this context, flocculation prior to sedimentation can enhance the settling rate, cell recovery and the final algal sludge concentration.

Flotation: Flotation is based on the lower density of the microalgae particles in comparison with water promoted by the adhesion of microscopic air bubbles, which raise microalgae to the surface where they can be separated via skimming [145]. This method is classified according to the mechanism of bubble production into dissolved air (DAF), dispersed air (DiAF) and electrolytic-flotation (EF) [141]. DAF consists on the formation of air bubbles by a sudden decompression of air-saturated water in the flotation tank at atmospheric pressure (Fig. 13b). In DiAF, air is sparged directly into the flotation tank through a diffuser or a high-speed mechanical agitator. On the other hand, EF involves the electrolysis of water into oxygen and hydrogen microbubbles. Although flotation is faster and can achieve higher biomass concentration (up to 7%) than sedimentation, this method typically involves a high energy demand [143]. Moreover, the use of additives is necessary to avoid the electrostatic repulsion between the gas bubbles and microalgae cells [146].

Centrifugation: This technology uses a centrifugal force to intensify the separation of microalgae from the medium based on their different density (Fig. 13c). Two types of centrifuges are widely applied for microalgae harvesting: disk stack centrifuges are suitable for separating particles of low size (3-30 μm) and dilute microalgae cultures (0.02-0.05%), while decanters are more appropriate for particle size greater than 15 μm and concentrated suspensions (>15%) [142]. Centrifugation is a fast and an efficient method that can achieve recovery efficiencies >90% and biomass concentrations up to 22% of solids without the addition of chemicals [141]. However, centrifugation is not cost-competitive for large-scale microalgae harvesting during wastewater treatment since it exhibits high investment costs and a prohibitive energy demand, thereby limiting its use only to the production of high-value compounds or as a second dewatering step. In addition, centrifugation can result in cell damage due to the high shear forces applied [147].

Filtration-based technologies: This harvesting method uses a permeable medium such as screens or membranes where microalgae are retained. A pressure difference across the barrier via vacuum, pressure or gravity force is necessary to force the liquid pass through. The system can operate in continuous or discontinuous mode in dead-end (the liquid flow is perpendicular to the filter surface) or cross-flow (the liquid flow is

parallel to the filter surface) configuration, the latter being preferred since cake formation is prevented (Fig. 13d) [144]. Membrane filters can be classified by the pore size into macrofiltration (>10 μm), microfiltration (0.1-10 μm) and ultrafiltration (0.001-0.10 μm). Despite lower pore sizes increase the efficiency of the separation, the energy required for microalgae separation increases proportionally. In this context, microfiltration exhibits the most appropriate size to retain most microalgae species at lower energy consumption than ultrafiltration [145]. Although nearly 100% of the microalgae can be retained in micro or ultrafiltration without addition of chemicals and no biomass disruption, the main drawback of filtration methods is the clogging and fouling of the membrane pores, which entails high maintenance costs to wash or replace the membranes [144].

Flocculation: Microalgae cells have a negative surface charge due to the presence of proton-active functional groups (i.e. carboxylic acids) that prevent the spontaneous aggregation of the cells as a result of electrostatic repulsive forces [144]. During flocculation, the addition of chemicals overcomes the electrostatic repulsion of microalgal cells, thus inducing the formation of large microalgae flocs. Flocculation can be induced by different mechanisms: i) charge neutralization, where the negative surface charge of microalgae is cancelled by the adsorbed positively charged ions, polymers or colloids; ii) electrostatic patch, where a positively charged polymer locally reverse the charge of the microalgae surface resulting in the connection of particles through patches with opposite charge; iii) bridging, where a polymer or colloid attaches simultaneously to the surface of several microalgae cells forming a bridge between them; and iv) sweeping, where the flocculant forms a precipitate that entangles microalgal cells (Fig. 14) [148]. A wide variety of flocculants are commercially available, the most applied being inorganic metal salts ($FeCl_3$, $Al_2(SO_4)_3$, $Fe_2(SO_4)_3$) and synthetic polyacrylamide-based polymers. Currently, natural biopolymers (i.e. chitosan, tanfloc, derivatives of cassia gum, cellulose or starch) are attracting interest as flocculants due to their biodegradability and sustainability [149]. On the other hand, flocculation can occur spontaneously by increasing the pH above 9, which results in salt precipitation (autoflocculation), or by the presence of other microorganisms (bioflocculation) [141,150]. Flocculation results in a rapid flocs separation by gravity or filtration-based technologies, which entails lower operational and/or investment costs. However, this harvesting technique requires the use of chemicals, which eventually can

result in contamination of the medium. In this context, the ideal flocculant should be inexpensive, efficient at low concentrations, non-toxic and environmentally friendly [142].

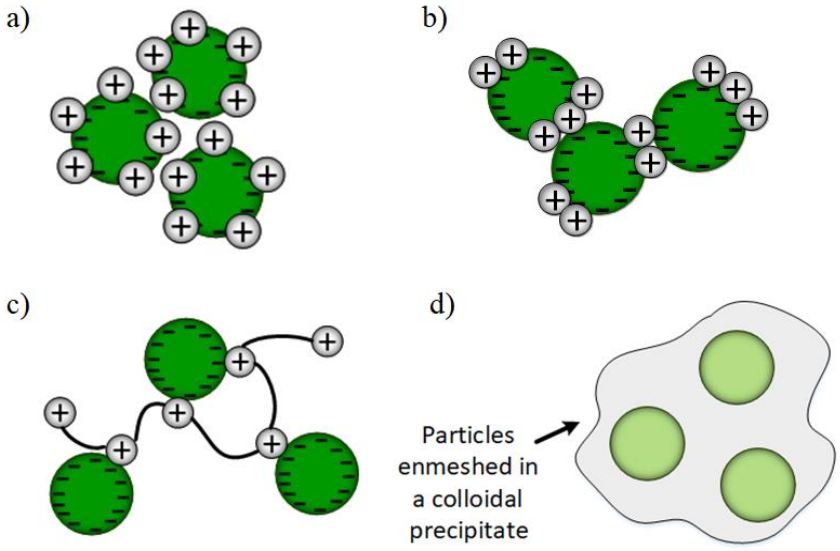

Fig. 14. Overview of different flocculation mechanisms: a) charge neutralization, b) electrostatic patch, c) bridging and d) sweeping flocculation. Adapted from Roselet et al. [141].

1.4. References

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Chapter 2

Aims and scope of the thesis

2.1. Justification of the thesis

The rapid increase of the energy demand worldwide as a result of the steady growth of the human population, together with the new energy and climate change policies focused on reducing greenhouse gas (GHG) emissions, have promoted the use of renewable energy sources. In this context, biogas from the anaerobic digestion of organic waste constitutes a promising biofuel able to reduce our current dependence on fossil fuels. In fact, the global biogas sector is growing based on the new business opportunities brought about by its potential use as a vehicle fuel or its injection into natural gas grids after biogas upgrading to biomethane. Despite the potential of biomethane as a renewable energy vector able to decrease fossil fuels consumption and GHG emissions, the current physical-chemical biogas upgrading technologies entail a high energy or chemical demand, which limits the environmental benefits of biomethane.

Photosynthetic biogas upgrading has emerged as an inexpensive and environmentally friendly alternative capable of removing CO_2 and H_2S from biogas and partially mitigating the eutrophication potential of wastewaters or digestates simultaneously. Despite these advantages, further research focused on the optimization of this technology is required in order to overcome the current technological and microbiological bottlenecks limiting its applicability, such as the limited CO_2 gas-liquid mass transfer rates and the subsequent biological CO_2 uptake by microalgae. Likewise, this innovative biotechnology must be further validated at demo scale prior to its full-scale implementation. Moreover, the development and validation of a control strategy to assure a consistent biomethane quality regardless of environmental conditions or operational failures is necessary in order to foster the acceptance of this biotechnology by the industrial sector. Finally, since algal-bacterial biomass harvesting constitutes a critical step in this microalgae-based process, the development of an efficient and low-cost biomass separation process prior use of the cultivation broth as scrubbing solution is a requirement. Therefore, more research in the above mentioned fields is still needed to consolidate the implementation of this promising green upgrading technology.

2.2. Main objectives

The overall objective of this thesis was to evaluate and optimize photosynthetic biogas upgrading in a high rate algal pond (HRAP) interconnected to an absorption column via a biomass settler, to obtain a biomethane complying with national and international

standards while recovering nutrients from domestic wastewater or digestates, and its subsequent implementation at semi-industrial scale with a robust control strategy. More specifically, the individual objectives pursued to achieve this overall goal were:

1. Evaluation of the influence of environmental conditions in the cultivation broth (i.e. alkalinity, temperature) on the final biomethane quality.
2. Evaluation of the long-term impact of high alkalinity on the robustness and efficiency of biogas upgrading process and microalgae activity.
3. Optimization of the process at semi-industrial scale under outdoors conditions.
4. Design and evaluation of a control strategy to maintain biomethane quality over time under typical operational fluctuations and failures during photosynthetic biogas upgrading at pilot scale and its subsequent validation at semi-industrial scale under outdoors conditions.
5. Enhancement of biomass harvesting in photosynthetic biogas upgrading via flocculation.

2.3. Development of the thesis

In the present thesis, the optimization and scale-up of a photosynthetic biogas upgrading process consisting of a HRAP interconnected to a biogas absorption column were conducted.

In order to fulfill the first objective aforementioned, the influence of inorganic carbon (IC) concentrations typically encountered in high and medium strength digestates and domestic wastewater, and temperatures representative of spring-autumn and summer seasons in temperate climates, on CO_2 and H_2S removal from biogas was investigated at pilot scale (180 L HRAP and 2.5 L absorption column) under indoor conditions (**Chapter 3**). Since a high alkalinity in the cultivation broth was previously identified as a key parameter to maintain the pH along the absorption column but also potentially detrimental to algal-bacterial activity, the impact of long-term process operation under high IC concentrations in the cultivation broth on H_2S and CO_2 removal efficiency and process robustness was assessed. CO_2 stripping from the HRAP to the atmosphere was also determined in order to evaluate the environmental sustainability of this technology at a high alkalinity. In addition, the influence of the biomass concentration in the cultivation broth on the performance of the upgrading process was also assessed (**Chapter 4**).

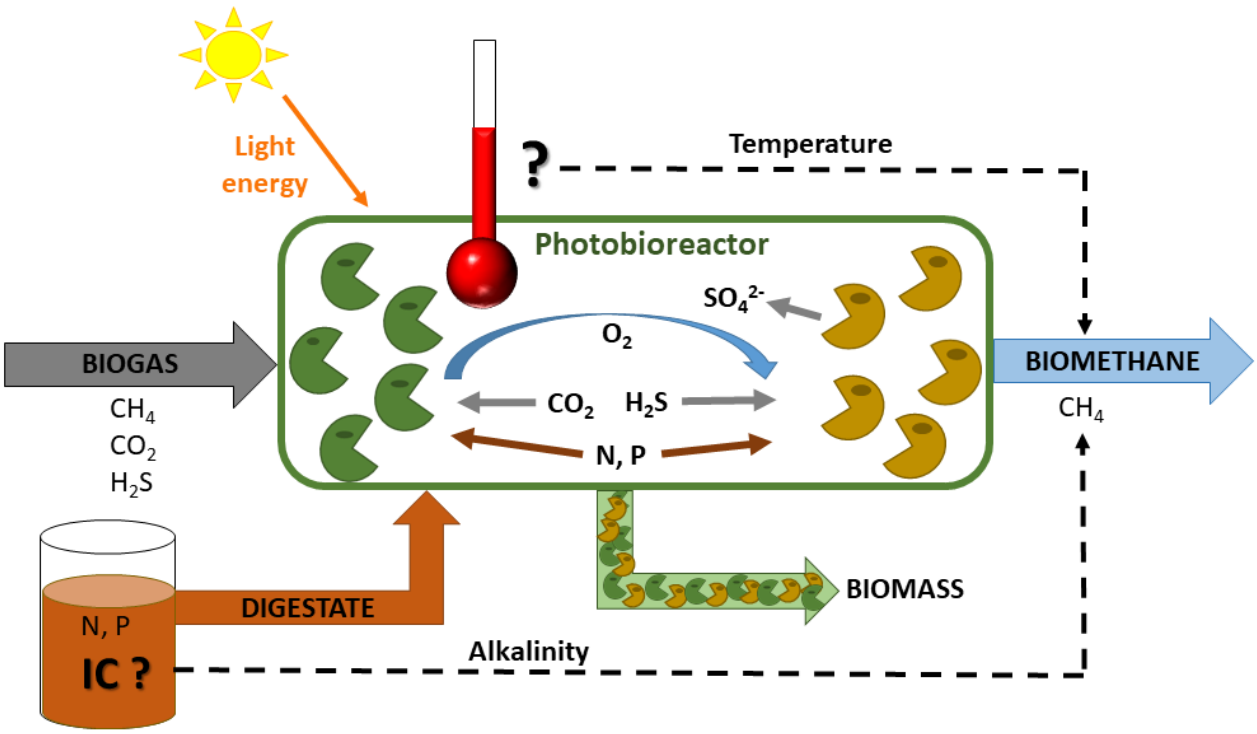
The scale-up of the process was carried out in a 9.6 m^3 HRAP interconnected to a 150 L absorption column via an external liquid recirculation of the supernatant from a 7 m^3 settler in the facilities of "El Torno" WWTP (Chiclana de la Frontera, Spain). An optimization of the liquid to biogas (L/G) ratio and biogas flowrate in the absorption column, the hydraulic retention time (HRT) in the HRAP and the nutrient source (domestic wastewater or centrate) was systematically performed (**Chapter 5**). Based on the previous results, the recycling liquid flowrate, which determines the L/G ratio in the absorption column, was chosen as manipulated variable to design a rule-based control strategy to cope with the fluctuations in the process over time. The performance of the control system against fluctuations in the biogas flowrate under different environmental conditions (pH, alkalinity and temperature) was evaluated at pilot scale (**Chapter 6**). The successful control strategy developed in Chapter 6 was further validated against environmental and operational variations (different pH of the cultivation broth, daily biogas production fluctuations) or operational failures at semi-industrial scale under outdoors conditions (**Chapter 7**).

Finally, the poor efficiency of settling of the algal-bacterial biomass in the settler located between the absorption column and the HRAP, which decreased the CO_2 and H_2S mass transfer efficiency in the absorption column, fostered research on the performance of different flocculants to promote biomass aggregation. The potential of this biomass harvesting technology was evaluated at lab scale in the facilities of the Aquatic Biology Lab at KU Leuven Campus Kulak (Kortrijk, Belgium) (**Chapter 8**).

Chapter 3

Influence of alkalinity and temperature on photosynthetic biogas upgrading efficiency in high rate algal ponds

Rodero, M. del R., Posadas, E., Toledo-Cervantes, A., Lebrero, R., Muñoz, R., 2018. Algal Res. 33, 284–290. doi:10.1016/j.algal.2018.06.001



Influence of alkalinity and temperature on photosynthetic biogas upgrading efficiency in high rate algal ponds

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ABSTRACT

Algal-bacterial photobioreactors have emerged as a cost-effective platform for biogas upgrading. The influence on biomethane quality of the inorganic carbon concentration (1500, 500 and 100 mg L^{-1}) and temperature (12 and 35ºC) of the cultivation broth was evaluated in a 180 L high rate algal pond (HRAP) interconnected to a 2.5 L absorption column via settled broth recirculation. The highest CO_2 and H_2S removal efficiencies (REs) from biogas were recorded at the highest alkalinity (CO_2-REs of 99.3±0.1 and 97.8±0.8% and H_2S-REs of 96.4±2.9 and 100±0% at 12 and 35ºC, respectively), which resulted in CH_4 concentrations of 98.9±0.2 and 98.2±1.0% at 12 and 35ºC, respectively, in the upgraded biogas. At the lowest alkalinity, the best upgrading performance was observed at 12ºC (CO_2 and H_2S-REs of 41.5±2.0 and 80.3±3.9%, respectively). The low recycling liquid to biogas ratio applied (0.5) resulted in a negligible O_2 stripping regardless of the alkalinity and temperature, which entailed a biomethane O_2 content ranging from 0 to 0.2±0.3%.

Keywords: algal-bacterial photobioreactor; alkalinity; biogas upgrading; biomethane; temperature.

1. Introduction

Biogas from the anaerobic digestion of organic matter constitutes a promising renewable energy vector for the production of heat and power in households and industry [1]. Raw biogas is mainly composed of CH_4 (40-75%), CO_2 (25-50%) and other components at lower concentrations such as H_2S (0.005-2%), oxygen (0-1%), nitrogen (0-2%), siloxanes (0-0.02%), ammonia (<1%) and halogenated hydrocarbons (VOC<0.6%) [2]. The high content of CO_2 significantly reduces the specific calorific value of biogas, increases its transportation costs and promotes emissions of CO and hydrocarbons during combustion. On the other hand, H_2S is a toxic and malodorous gas that severely reduces the lifespan of the biogas storage structures, pipelines, boilers and internal combustion engines [3]. The removal of these biogas pollutants is mandatory in order to comply with the technical specifications required for biogas injection into natural gas grids (CH_4 > 95%, CO_2 < 2.5-4%, O_2 < 0.001-1% and H_2S + COS < 5 mg/Nm^3) or use as a vehicle fuel [4]. State-of-the-art physical/chemical or biological technologies for CO_2 removal often need a previous H_2S cleaning step, while the few technologies capable of simultaneously removing CO_2 and H_2S from biogas (i.e. water/chemical scrubbing and membrane separation) exhibit a high energy and chemicals consumption, which limits their economic and environmental sustainability for biogas upgrading [5]. In this context, algal-bacterial symbiosis represents a cost-effective and environmentally friendly platform for the simultaneous removal of CO_2 and H_2S from raw biogas in a single step process [6].

Photosynthetic biogas upgrading in algal-bacterial photobioreactors is based on the light-driven CO_2 consumption by microalgae coupled to the oxidation of H_2S to either elemental sulfur or sulfate by sulfur-oxidizing bacteria (i.e. belonging to the Thioalbus genus) using the oxygen photosynthetically produced [3, 7]. The environmental and economic sustainability of the process can be boosted with the integration of wastewater treatment in the photobioreactor devoted to biogas upgrading [8]. In this regard, digestate or domestic wastewater can be used as an inexpensive nutrient source for microalgae and bacteria growth during photosynthetic biogas upgrading, which in turn would reduce the costs associated to nutrients removal [9,10]. Recent investigations have focused on the optimization of the simultaneous biogas upgrading and digestate treatment in photobioreactors. These studies have identified the optimum photobioreactor configuration [6,8,11,12], the strategies for minimizing oxygen

concentration in the biomethane [13,14] and the influence of light intensity, wavelength and photoperiod regime on the final quality of the upgraded biogas under indoors conditions [15–19]. Unfortunately, most of these previous works did not result in a biomethane composition complying with the specifications of most European regulations due to the limited CO_2 mass transfer rates from the raw biogas to the aqueous phase [20]. In this context, a recent study conducted outdoors in a high rate algal pond (HRAP) interconnected to an external absorption column for the simultaneous treatment of biogas and centrate suggested that both alkalinity and temperature in the algal-bacterial broth can play a key role on the final biomethane quality [11]. Indeed, culture broth alkalinity determines the kinetics of both microalgae growth in the HRAP and CO_2/H_2S absorption in the absorption column [21]. Likewise, culture broth temperature directly impacts on the gas/liquid equilibria and biomass growth kinetics [19]. However, despite the relevance of these environmental parameters on the performance of photosynthetic biogas upgrading, no study has evaluated to date the effect of alkalinity and temperature on the final quality of biomethane in algal-bacterial photobioreactors.

This work systematically evaluated the influence of inorganic carbon concentration and temperature in the cultivation broth on biomethane quality in a 180 L HRAP interconnected to a 2.5 L absorption column via external recirculation of the settled cultivation broth under indoor conditions. The tested inorganic carbon concentrations (1500, 500 and 100 mg L^{-1}) are typically encountered in high and medium strength digestates and domestic wastewater, respectively, while the tested temperatures are representative of spring-autumn (12 ºC) and summer (35 ºC) seasons in temperate climates.

2. Materials and methods

2.1. Biogas and centrate

A synthetic gas mixture composed of CO_2 (29.5%), H_2S (0.5%) and CH_4 (70%), was used in this study as a model biogas (Abello Linde; Spain). Centrate was collected from the anaerobically digested sludge-dehydrating centrifuges at Valladolid wastewater treatment plant (WWTP) and stored at 4 ºC prior to use. The average centrate composition was as follows: inorganic carbon (IC) = 459 ± 83 mg L^{-1}, total nitrogen (TN) = 576 ± 77 mg L^{-1} and $S\text{-}SO_4^{2-}$ = 4.7 ± 3.4 mg L^{-1}. NH_4Cl was added to the raw

centrate to a final TN concentration of 1719±235 mg L^{-1} in order to simulate a high-strength digestate and thus minimize the flow rate of centrate used in the pilot plant.

2.2. Experimental set-up

The experimental set-up was located at the Department of Chemical Engineering and Environmental Technology at Valladolid University (Spain). The set-up consisted of a 180 L HRAP (depth: 15 cm, width: 63 cm, length: 202 cm) with an illuminated surface of 1.2 m^2 divided by a central wall in two water channels. The HRAP was interconnected to a 2.5 L absorption column (Ø: 4.4 cm, height: 165 cm) via external liquid recirculation of the supernatant of the algal-bacterial cultivation broth from a 10 L conical settler coupled to the HRAP (Figure 1). The remaining algal bacterial biomass collected at the bottom of the settler was continuously recirculated to the HRAP in order to avoid the development of anaerobic conditions in the settler due to an excessive biomass accumulation. The HRAP cultivation broth was continuously agitated by a 6-blade paddlewheel at an internal recirculation velocity of ≈ 20 cm s^{-1}. A photosynthetic active radiation (PAR) of 1350 ± 660 µmol m^{-2} s^{-1} at the HRAP surface was provided by six high-intensity LED PCBs (Phillips SA, Spain) operated in a 12h:12h light/dark regime.

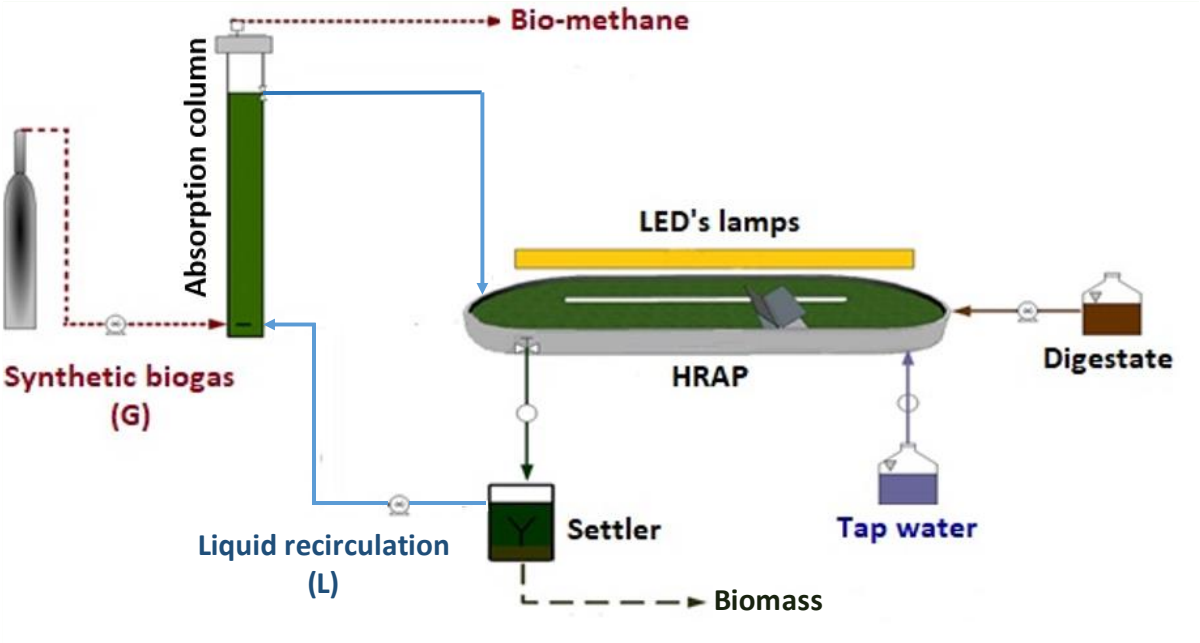

Figure 1. Schematic diagram of the experimental set-up.

2.3. Operational conditions

Six operational conditions were tested in order to assess the influence of alkalinity and temperature on biomethane quality. The influence of IC concentrations of 1500, 500 and 100 mg L^{-1} was evaluated in stages I-II, III-IV and V-VI, respectively, while a temperature of 35 ºC was maintained during stages I, III and V and a temperature of 12ºC during stages II, IV and VI (Table 1). The HRAP was initially filled with an aqueous solution containing a mixture of $NaHCO_3$ and Na_2CO_3 before inoculation to adjust the initial IC concentration to the corresponding concentration set in the operational stage. The IC concentration of the digestate fed to the HRAP during each operational stage was also adjusted accordingly. Thus, IC concentrations of 1500 and 500 mg L^{-1} were obtained by addition of $NaHCO_3$ to the raw centrate, while IC concentrations of 100 mg L^{-1} were achieved via an initial centrate acidification with HCl aqueous solution (37%) to a final pH of 5.5 in order to remove IC by air-aided CO_2 stripping followed by $NaHCO_3$ addition to adjust the IC concentration. The temperature of the HRAP cultivation broth was controlled with an external heat exchanger (Fisherbrand™ Polystat™ Immersion Circulator, Germany). A consortium of microalgae/cyanobacteria (from now on referred to as microalgae) from outdoors HRAPs treating centrate and domestic wastewater at the Department of Chemical Engineering and Environmental Technology at Valladolid University and at the WWTP of Chiclana de la Frontera (Spain), respectively, was used as inoculum in each operational stage.

Table 1. Average environmental parameters along with the corresponding standard deviation (n=4) in the HRAP, absorption column and digestate under steady state conditions during the six operational stages tested.

Stage	**I**	**II**	**III**	**IV**	**V**	**VI**
Average IC feed (mg L^{-1})	1581±135	1467±115	505±57	517±46	102±7	103±11
Average Temperature (ºC)	35.0±1.3	12.5±1.8	36.0±1.2	12.4±2.0	36.0±1.6	12.9±1.8
Evaporation rate (L m^{-2} d^{-1})	14.1±0.2	2.3±0.4	15.8±1.1	1.6±0.3	17.5±0.1	1.8±0.3
DO light (mg L^{-1})	10.1±2.1	14.4±0.9	13.5±0.8	16.6±1.9	8.8±0.8	16.5±1.7
DO dark (mg L^{-1})	1.3±0.0	6.2±1.2	3.7±0.1	7.0±0.9	4.6±0.6	10.0±0.5
pH HRAP	11.0±0.0	10.5±0.3	10.5±0.4	9.7±0.2	7.2±0.3	7.5±0.2
pH outlet column	10.4±0.1	9.9±0.2	7.3±0.1	6.9±0.1	5.3±0.2	5.5±0.1
Average IC HRAP (mg L^{-1})	1667±157	1891±31	321±52	367±23	4±1	7±2
TSS (g L^{-1})	0.43±0.02	0.54±0.05	0.44±0.07	0.45±0.02	0.20±0.07	0.18±0.03
S-SO_4^{2-} accumulation (g m^{-3} d^{-1})	1.85	1.10	1.57	0.97	1.33	0.60
Duration (d)	26	28	29	27	28	26

IC: inorganic carbon; DO: dissolved oxygen; TSS: total suspended solids

During the illuminated periods, the HRAP was fed with the modified digestate as a nutrient source at a flow rate of 2 L d^{-1}, while synthetic biogas was sparged into the absorption column under co-current flow operation at a flow rate of 4.9 L h^{-1} and a recycling liquid flow rate (L min^{-1}) to biogas flow rate (L min^{-1}) ratio (L/G, dimensionless) of 0.5 [12]. Tap water was continuously supplied in order to compensate water evaporation losses. A biomass productivity of 7.5 g dry matter m^{-2} d^{-1} was set in the six operational stages evaluated by controlling the biomass harvesting rate. The algal-bacterial biomass was harvested by sedimentation after coagulation-flocculation via addition of the polyacrylamide-based flocculant Chemifloc CV-300 (Chemipol S.A) [22]. This operational strategy resulted in a process operation without effluent. Approximately two weeks after the beginning of each stage, the system had already achieved a steady state, which was confirmed by the negligible variation of most parameters during the rest of the stage (variations < 5% of the recorded values).

2.4. Sampling procedure

The ambient and cultivation broth temperatures, the flow rates of digestate, tap water and external liquid recycling, and the dissolved oxygen (DO) concentration in the cultivation broth were monitored three times per week during the illuminated and dark periods. The PAR was measured at the HRAP surface at the beginning of each stage. Gas samples of 100 μL from the raw and upgraded biogas were drawn three times per week in order to monitor the CO_2, H_2S, CH_4, O_2 and N_2 concentrations. The inlet and outlet biogas flow rates at the absorption column were also measured to accurately determine CO_2 and H_2S removals. Liquid samples of 100 mL of digestate and cultivation broth were drawn three times per week and filtered through 0.20 μm nylon filters to monitor pH, dissolved IC, TN and SO_4^{2-}. In addition, liquid samples of 20 mL were also drawn three times per week from the cultivation broth to monitor the TSS concentration. Unfortunately, no analysis of the microbial population structure was conducted in this study.

2.5. Analytical methods

The DO concentration and temperature were monitored with an OXI 330i oximeter (WTW, Germany), while a pH meter Eutech Cyberscan pH 510 (Eutech instruments, The Netherlands) was used for pH determination. The PAR at the HRAP surface was recorded with a LI-250A lightmeter (LI-COR Biosciences, Germany). CO_2, H_2S, O_2, N_2 and CH_4 gas concentrations were analysed using a Varian CP-3800 GC-TCD (Palo

Alto, USA) according to Posadas et al. [13]. The dissolved IC and TN concentrations were determined using a Shimadzu TOC-VCSH analyser (Japan) equipped with a TNM-1 chemiluminescence module. SO_4^{-2} concentration was measured by HPLC-IC according to Posadas et al. [23], while the determination of TSS concentration was carried out according to standard methods [24] .

2.6. Statistical treatment

The ambient and cultivation broth temperatures, pH, cultivation broth TSS concentrations, the flow rates of digestate, tap water and external liquid recycling, the dissolved oxygen (DO) concentration, and the flowrate and composition of biogas were obtained under steady state operation. CO_2-REs and H_2S-REs were calculated according to [13] based on duplicate measurements of the biogas and biomethane composition. The results here presented were provided as the average values (obtained for at least 4 sampling days over a two weeks period during each steady state) along with their corresponding standard deviation.

A t-student statistical analysis was performed in order to determine the statistically significant differences between the pH value at the bottom and the top of the absorption column. In addition, the t-student test was applied to determine the effect of temperature at the different alkalinities tested. Finally, a one-way ANOVA was performed to determine the effect of alkalinity and temperature on the quality of the biomethane produced along the six operational stages.

3. Results and discussion

3.1. Environmental parameters and biomass concentration

The average water loss by evaporation in the HRAP (average tap water flow rate needed to maintain the level of the HRAP constant) during process operation at 35 ºC was 15.9±1.2 L d^{-1} m^{-2}, while this value decreased to 1.9±0.4 L d^{-1} m^{-2} at 12 ºC (Table 1). The maximum evaporation rate recorded in this study was ~1.8 times higher than the maximum reported by Posadas et al. [11] in a similar outdoors HRAP during summer in a temperate climate and ~2.6 times higher than the highest value estimated by Guieysse et al. [25] in an arid location. The high water losses here recorded were caused by the high and constant temperatures of the cultivation broth throughout the entire day (no decrease in the culture broth temperature occurred during the night) and the high turbulence induced by the oversized paddlewheel typical in lab-scale systems [25]. On

the other hand, the lower temperature prevented water losses, the minimum value recorded being in the range obtained by Posadas et al. [26] in a similar outdoors HRAP during spring in a temperate climate (3±8 L m^{-2} d^{-1}).

The average DO concentrations in the cultivation broth during the illuminated period (~6 hours after turning on the lights) were 10.1±2.1, 14.4±0.9, 13.5±0.8, 16.6±1.9, 8.8±0.8 and 16.5±1.7 mg O_2 L^{-1} during stages I, II, III, IV, V and VI, respectively; while the DO concentrations during the dark period (~6 hours after turning off the lights) averaged 1.3±0.5, 6.2±1.2, 3.7±0.1, 7.0±0.9, 4.6±0.6 and 10.0±0.5 mg O_2 L^{-1} in stages I to VI, respectively. The higher DO concentrations recorded at 12 ºC were attributed to the increased oxygen solubility at low temperatures [27]. No pernicious effect of these DO concentrations on microalgae activity was expected since inhibition of photosynthesis typically occurs above 25 mg O_2 L^{-1}, and the values remained within the optimal range to support nutrients and CO_2 bioassimilation [28].

The average pHs in the HRAP during stages I, II, III, IV, V and VI were 11.0±0.0, 10.5±0.3, 10.5±0.4, 9.7±0.2, 7.2±0.3 and 7.5±0.2, respectively. These findings confirmed that the influence of the IC concentration in the cultivation broth was higher than that of the temperature on the steady state pH of the cultivation broth, which was in accordance with previous results from Posadas et al. [11]. Moreover, the highest pH values here recorded matched those observed by Toledo-Cervantes et al. [12] during the simultaneous treatment of biogas and digestate in a similar experimental set-up, while Lebrero et al. [20] reported comparable pHs to the lowest values obtained in this study when evaluating biogas upgrading in a transparent PVC column photobioreactor. A higher pH in the cultivation broth enhances the mass transfer rate of the acidic gases (CO_2 and H_2S) from biogas to the liquid phase, which ultimately results in higher upgrading performances as discussed below [6].

TSS concentrations of 0.4-0.5 g L^{-1} were recorded during process operation at both high and medium alkalinity (Table 1). Thus, the biomass concentration in the cultivation broth at the imposed biomass productivity (7.5 g dry matter m^{-2} d^{-1}) during stages I to IV was representative of the operation of conventional outdoor raceways, where TSS concentration typically ranges from 0.3 to 0.5 g L^{-1} [29]. However, the biomass concentration and productivity, during stages V and VI (IC concentration of 100 mg L^{-1}), decreased to 0.2 g TSS L^{-1} and 5-7 g dry matter m^{-2} d^{-1} respectively, due to the lower

carbon load supplied in the feed and the lower CO_2 mass transfer in the absorption column mediated by the low pH of the cultivation broth (as discussed in section 3.2.1).

3.2. Biogas upgrading efficiency

3.2.1. CO_2- removal efficiency

Average CO_2-REs of 99.3±0.1, 97.8±0.8, 48.3±3.6, 50.6±3.0, 30.8±3.6 and 41.5±2.0% were recorded during stages I, II, III, IV, V and VI, respectively (Figure 2).

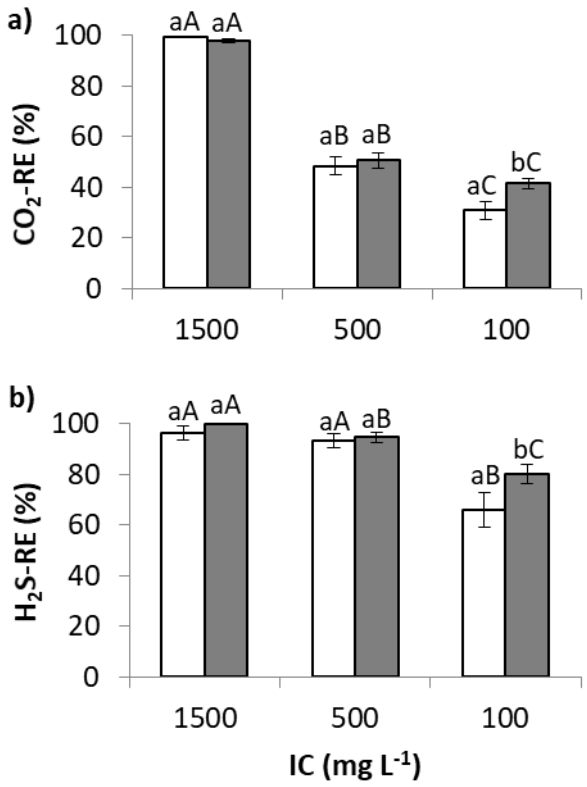

Figure 2. Influence of the inorganic carbon concentration (IC) and temperature on the removal efficiency (RE) of a) carbon dioxide (CO_2) and b) hydrogen sulphide (H_2S) at 35ºC (□) and at 12ºC (■), average removal efficiencies and their standard deviation (n=8). Similar lowercase letters indicate no significant differences ($p>0.05$) when comparing both temperatures at each IC concentration. Similar uppercase letters indicate no significant differences ($p>0.05$) when comparing the IC concentrations at the same temperature.

During stages I and II (1500 mg IC L^{-1}), the high CO_2 mass transfer rates between the biogas and the liquid phase were promoted by the high pH (> 10.5) and high buffer capacity of the cultivation broth. The initial pH of the system (pH = 10.5) was roughly maintained in the cultivation broth of the HRAP (10.4±0.1) and along the absorption column (9.9±0.2) as a result of the high alkalinity of the digestate (Table 1). During stages III and IV (500 mg IC L^{-1}), a slight decrease in the pH of the cultivation broth from the initial value occurred as a result of biogas absorption in the column due to both

the acidic nature of CO_2 and H_2S and the lower buffer capacity of the media, thus resulting in lower CO_2-REs. This effect was more pronounced in stages V and VI (100 mg IC L^{-1}), where the low buffer capacity of the cultivation broth was unable to maintain a constant and high pH, which resulted in the lowest CO_2-REs recorded in this experiment (Table 1). The pH of the cultivation broth significantly differed (t-student test, $p < 0.05$) between the bottom (10.5±0.4, 9.7±0.2, 7.2±0.3 and 7.5±0.2 in stages III, IV, V and VI, respectively) and the top (7.3±0.1, 6.9±0.1, 5.3±0.2 and 5.5±0.1 in stages III, IV, V and VI, respectively) of the absorption column at medium and low alkalinity (Table 1). Higher L/G ratios would have avoided these high pH variations along the absorption column. Nevertheless, a lower biomethane quality would be expected at high L/G ratios as a result of the enhanced O_2 and N_2 stripping from the recycling cultivation broth to the upgraded biogas [8]. These data was in accordance to Lebrero et al. [20], who reported an average CO_2-RE of 23% at a pH 7 and of 62% when the pH of the cultivation broth was increased up to 8.1. Overall, these results showed the relevance of inorganic carbon concentration to maintain a high pH in the scrubbing cultivation broth during biogas upgrading.

On the other hand, a negligible effect of the temperature on CO_2-RE was found at high and medium alkalinity (from stages I to IV) (Figure 2). However, the higher CO_2 solubility at lower temperatures resulted in a higher CO_2-RE at 12ºC compared to that achieved at 35ºC under low alkalinity (stages V and VI) (Figure 2). This suggests that, despite the lower alkalinity of the cultivation broth could be partially compensated with the decrease in temperature, the latter mediated a major effect on CO_2 mass transfer.

C-CO_2 desorption ratios, defined as the ratio between the mass flow rate of IC desorbed from the cultivation broth and the total mass flow rate of IC supplied to the system (C-CO_2 absorbed in the absorption column + IC supplied in the centrate) and considering a carbon content of 50% in the microalgal biomass [30], of 51, 50, 2 and 4% were recorded in stages I, II, III and IV, respectively. However, a negligible C-CO_2 desorption was estimated at low alkalinities as a result of the low CO_2 mass transfer in the absorption column and low IC input via centrate addition, which ultimately resulted in process operation under carbon limiting conditions (Table 2). The highest CO_2 desorption rates obtained during stages I and II were associated to the high IC concentration in the cultivation broth, which supported a positive CO_2 concentration gradient to the atmosphere even though IC was mainly in the form of CO_3^{2-}. On the

contrary, IC was preferentially used by microalgae rather than removed by stripping despite the low pH prevailing in the cultivation broth at low alkalinity. These results agreed with those reported by Meier et al. [19], who identified stripping as the main mechanism responsible for carbon removal in a 50 L photobioreactor fed with a mineral medium and connected to a bubble column. Similarly, Alcántara et al. [10] observed a 49% CO_2 loss by desorption in a comparable 180 L HRAP interconnected to an absorption column during the simultaneous treatment of biogas and centrate.

Table 2. Inorganic carbon mass balance with the corresponding standard deviation (n=4) under steady state conditions during the six operational stages tested.

STAGE	INPUTS (g d^{-1})		OUTPUTS (g d^{-1})		
	IC biogas[1]	IC digestate[1]	IC biomass[1]	IC accumulated[1]	IC desorption[2]
I	7.87±0.24	1.48±0.20	4.54±0.00	0.03±0.04	4.78±0.40
II	7.91±0.61	1.37±0.15	4.54±0.00	0.02±0.04	4.73±0.70
III	4.04±0.29	0.46±0.04	4.54±0.00	0.00±0.00	0.11±0.04
IV	4.20±0.32	0.45±0.05	4.54±0.00	0.00±0.00	0.20±0.23
V	2.78±0.46	0.08±0.01	2.91±0.00	0.00±0.00	0.00±0.00
VI	3.78±0.19	0.10±0.01	3.93±0.00	0.00±0.00	0.00±0.00

1-Measured; 2-Estimated from the mass balance

3.2.2. H_2S- removal efficiency

Average H_2S-REs of 96.4±2.9, 100±0, 93.4±2.6, 94.7±1.9, 66.2±6.9 and 80.3±3.9% were recorded during stages I, II, III, IV, V and VI, respectively (Figure 2). The higher H_2S-REs compared to CO_2-REs were attributed to the higher dimensionless Henry's Law constants of H_2S, defined as the ratio between the aqueous phase concentration of H_2S or CO_2 and its gas phase concentration (H_{H2S} ≈2.13 and H_{CO2}≈0.71 at 20ºC) [27]. The highest H_2S removals were achieved at the highest alkalinities (stages I and II), corresponding to the highest pH along the absorption column. Similarly, Franco-Morgado et al. [18] obtained H_2S-RE of 99.5±0.5% during the operation of a HRAP interconnected to an absorption column using a highly carbonated medium at a pH of 9.5. On the other hand, the low pH in the cultivation broth together with the large decrease in pH in the absorption column under low alkalinity caused the poor H_2S removal recorded (Table 1). These results were in accordance with those reported by Bahr et al. [6], who observed a significant deterioration in the H_2S-RE from 100% to 80% when the pH in the absorption column decreased from 7 to 5.4 in a similar HRAP-absorption column system.

No significant effect (t-student test, $p>0.05$) of the temperature was observed at high-medium alkalinity on the removal of H_2S (Figure 2). On the contrary, higher H_2S-REs were recorded at 12ºC under low alkalinity likely due to the increase in the aqueous solubility of H_2S.

H_2S oxidation ratios (defined as the mass flow rate of $S\text{-}SO_4^{2-}$ accumulation in the HRAP divided by the mass flow rate of $S\text{-}H_2S$ absorbed in the absorption column, subtracting the $S\text{-}SO_4^{2-}$ introduced with the centrate) of 100%, 87% and 94% were obtained at 35 ºC during stages I, III and V, respectively. However, an incomplete oxidation of H_2S occurred at 12ºC, resulting in ratios of 55%, 67% and 33% during stages II, IV and VI, respectively. The remaining sulphur being most likely present as S-intermediates (i.e S°, thiosulfate or sulfite) or biomass (a typical S content of 0.07% can be assumed). Incomplete H_2S oxidation was also reported by Toledo-Cervantes et al. [3], who estimated than only 40% of the absorbed H_2S was oxidized to SO_4^{2-} in a similar experimental set-up. Interestingly, the high DO concentrations in the cultivation broth at 12ºC did not result in higher H_2S oxidation ratios likely due to the lower microbial activity at low temperatures.

3.2.3. Biomethane composition

An average CH_4 content of 98.9±0.2, 98.2±1.0, 80.9±0.8, 82.5±1.2, 75.9±0.7 and 79.2±0.7% was obtained in the final biomethane during stages I, II, III, IV, V and VI, respectively (Figure 3). The high CH_4 contents in stages I and II (1500 mg IC L^{-1}) were attributed to the high absorption efficiency of CO_2 and H_2S and the limited desorption of N_2 and O_2. Furthermore, a negligible CH_4 absorption in the absorption column was observed along the six operational stages, with average losses of 2.8±3.4% (on a mass basis) regardless of the alkalinity or temperature. Posadas et al. [11] obtained slightly lower CH_4 losses (2.2±1.2%) in an outdoors HRAP, while CH_4 losses of 4.9±2.4% were reported by Toledo-Cervantes et al. [3] in a similar indoors system. At this point it should be pointed out that the composition of the biomethane produced in stages I and II complied with most European regulations for biogas injection into natural gas grids or use as autogas in terms of content of CH_4 (≥95%) and CO_2 < 2.5-4% [5]. In fact, the CO_2 content in the upgraded biogas accounted for 0.3±0.1, 0.9±0.3, 18.4±1.0, 16.9±0.8, 23.0±0.9 and 20.3±0.6% during stages I, II, III, IV, V and VI, respectively (Figure 3).

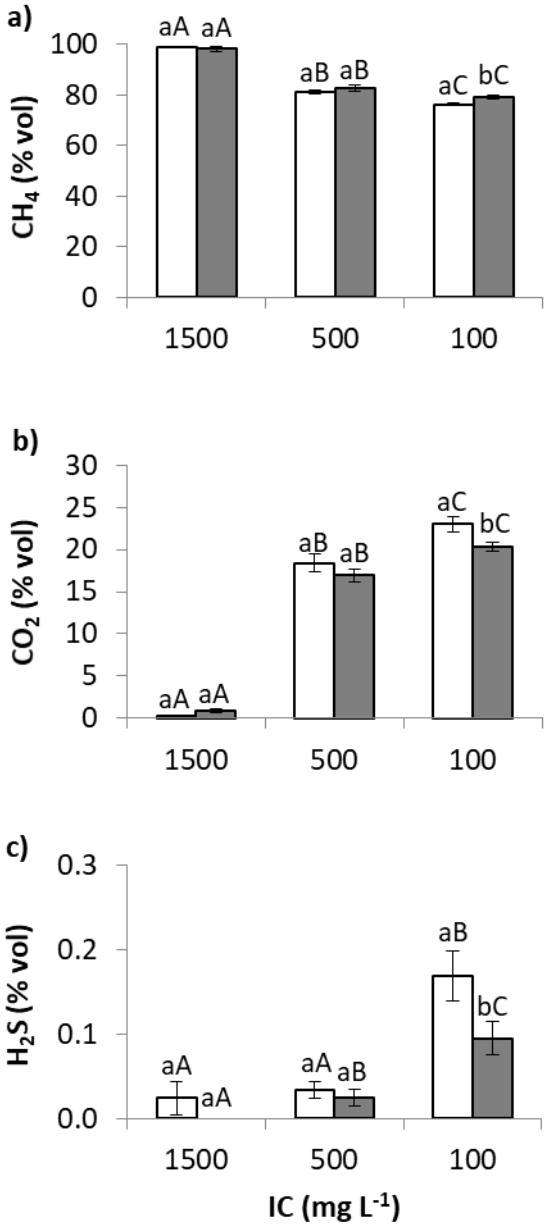

Figure 3. Influence of the inorganic carbon concentration (IC) and temperature on bio-methane composition: a) CH_4, b) CO_2, c) H_2S average concentrations and their standard deviation (n=8) at 35ºC (□) and at 12ºC (■). Same lowercase letters indicate not significantly different ($p>0.05$) when compare both temperature at each IC concentration. Same uppercase letters indicate no significantly different ($p>0.05$) when compare the IC concentration for the same temperature.

During stages I to IV, H_2S concentrations below 0.03% were recorded in the upgraded biogas, which complied with EU regulations (Figure 3). Moreover, no significant differences (One-way ANOVA, $p>0.05$) in O_2 and N_2 content of the upgraded biogas were observed during the six operational stages (O_2 concentrations of 0.0±0.0, 0.2±0.3, 0.0±0.0, 0.1±0.1, 0.2±0.1 and 0.1±0.2%, and N_2 concentrations of 0.7±0.2, 0.7±0.6, 0.7±0.3, 0.5±0.5, 0.8±0.4 and 0.3±0.3% during stages I, II, III, IV, V and VI,

respectively), which also matched the levels required by most European regulations (O_2 < 0.001-1%) (Figure 4). These results might be explained by the low L/G ratio (0.5) applied during the study, which entailed a limited O_2 and N_2 stripping from the cultivation broth to the biomethane in the absorption column [18]. No significant effect of the microalgae population structure on the removals of CO_2 and H_2S, and on the stripping of N_2 or O_2, was expected above a certain photosynthetic activity threshold. In our particular study, the control of the biomass productivity (fixed at 7.5 g m^{-2} d^{-1}) guaranteed a constant rate of photosynthetic activity along the process regardless of the microalgae species dominant. In addition, previous works have consistently reported no-correlation between the dominant microalgae species and biogas upgrading performance [3, 8, 12].

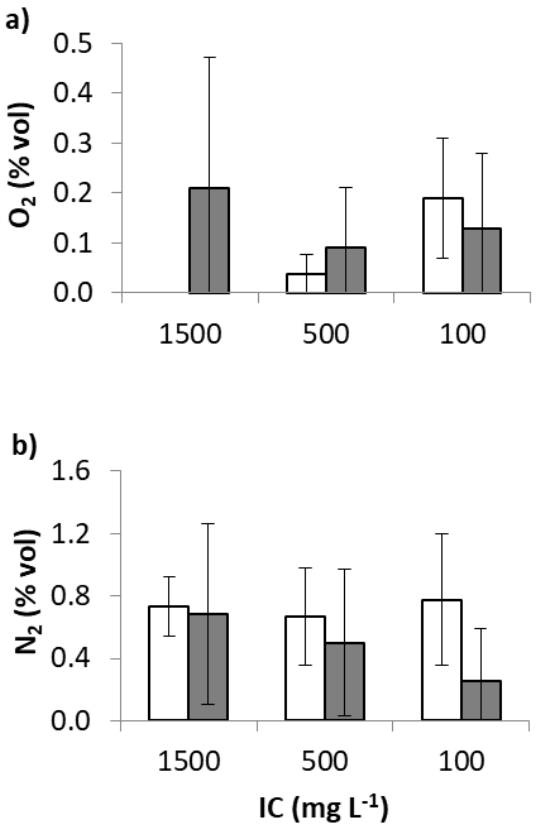

Figure 4. Influence of the inorganic carbon concentration (IC) and temperature on biomethane composition: a) O_2, b) N_2 average concentrations and their standard deviation (n=8) at 35ºC (□) and at 12ºC (■). Average values were not significantly different during the six operational stages (p>0.05).

4. Conclusions

The alkalinity of the cultivation broth was here identified as a key environmental parameter influencing biomethane quality. A negligible effect of the temperature on the

quality of the upgraded biogas was recorded at high-medium alkalinity, while temperature played a significant role on biomethane quality at low alkalinity. Biomethane composition complied with most European regulations for biogas injection into natural gas grids or use as a vehicle fuel when photosynthetic biogas upgrading was carried out at high alkalinity (IC concentrations of >1500 mg IC/L). In addition, this study also revealed that low alkalinity media might induce inorganic carbon limitation, which ultimately decreases the CO_2 mass transfer from biogas as a result of a rapid acidification of the scrubbing cultivation broth in the absorption column.

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Chapter 4

Long-term influence of high alkalinity on the performance of photosynthetic biogas upgrading

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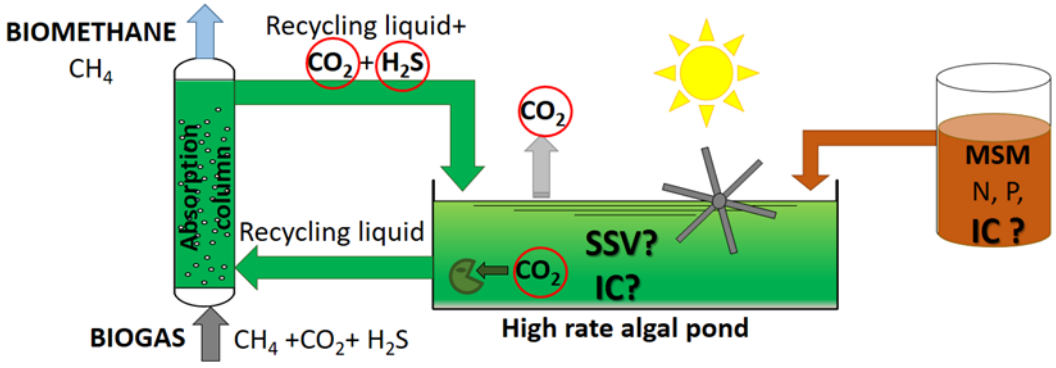

Long-term influence of high alkalinity on the performance of photosynthetic biogas upgrading

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ABSTRACT

The alkalinity of the cultivation medium plays a key role on photosynthetic biogas upgrading, exerting impact not only on the mass-transfer of CO_2 and H_2S in the biogas scrubbing column but also on the subsequent CO_2 uptake or stripping to the atmosphere. The long-term performance of algal-bacterial processes devoted to the concomitant removal of CO_2 and H_2S from biogas in a 180 L open pond interconnected to a 2.5 L biogas scrubbing column via an external liquid recirculation of supernatant from a 8 L conical settler under process operation at high inorganic carbon (IC) concentrations was assessed. The influence of biomass concentration in the cultivation medium on process performance was also evaluated. CO_2 concentrations in the upgraded biogas fluctuated between 1.5 and 4.4% at IC concentrations in the cultivation medium of 1200 mg C L^{-1}, and remained almost constant (0.7 ± 0.1%) at IC concentrations > 2400 mg C L^{-1}. However, the increase in the IC concentration from 1203 to 3476 mg C L^{-1} entailed an increase in C-CO_2 stripping from 14.5 to 33.4% of the IC input to the system. The increase in biomass concentration from 0.33 to 1.38 g SSV L^{-1} entailed a reduction in CO_2 removal of 1.1% even under process operation at high alkalinity. H_2S removal efficiencies of 100% were achieved regardless the IC or biomass concentration.

Keywords: algal-bacterial symbiosis; alkalinity; biomass concentration; biogas upgrading; biomethane.

1. Introduction

Biogas constitutes the most valuable byproduct from the anaerobic degradation of residual organic substrates. Typically, biogas consists of CH_4 (40-75%), CO_2 (25-50%), H_2S (0.005-3%) and other components such as O_2, N_2, NH_3, siloxanes, halogenated hydrocarbons and water at trace level concentrations [1]. The energy potential of biogas, due to its high CH_4 content, has promoted the use of this bioenergy source as a substitute of fossils fuels [2]. In this context, the global production of biogas has increased from 0.28 to 1.31 exajoule during the period 2000-2016, which represented a total volume of biogas of approx. 60.8 billion Nm^3 [3]. However, the presence of pollutants, such as CO_2 and H_2S, prevents the direct use of biogas as a vehicle fuel or its addition into natural gas networks, which requires concentrations of $CH_4 > 90\%$, $CO_2 <$ 2–4%, $O_2 < 0.001–1\%$ and $H_2S + COS < 5$ mg/Nm^3 according to most international regulations [4,5]. CO_2 removal increases the specific biogas energy content, reduces its transportation costs and results in lower greenhouse gas emissions during biogas combustion, while the removal of H_2S is crucial due to its hazardous, malodorous and corrosive nature [6,7].

Physical-chemical technologies including water/organic/chemical scrubbing, pressure swing adsorption and membrane separation for CO_2 removal, and *in situ* precipitation, adsorption on activated carbon or metal ions, absorption and membrane separation for H_2S removal are widely applied for biogas upgrading [8]. Nevertheless, most of these technologies are not able to support the simultaneous removal of both components and typically entail a high energy and chemical consumption, which limit the environmental and economic sustainability of biomethane [9]. Likewise, biological technologies (i.e. biological methanation of CO_2 with H_2 and biofiltration or *in situ* microaerobic digestion for H_2S removal) must be combined to remove CO_2 and H_2S from biogas [10]. In this context, biogas upgrading based on algal–bacterial symbiosis is a cost-competitive alternative for the concomitant removal of H_2S and CO_2 from biogas in an environmentally sustainable way [11]. This platform technology is based on the light-driven CO_2 uptake by microalgae and the oxidation of H_2S to S^0/SO_4^{2-} by sulfur-oxidizing bacteria promoted by the oxygen photosynthetically generated [12]. In addition, the liquid fraction of digestates from anaerobic digestion can be used as a free water and nutrient source to support algal-bacterial growth, which represents an economic and environmental benefit of this technology compared to its

physical/chemical and biological counterparts [13].

Recent works have evaluated the influence of operational and environmental parameters such as the wavelength, intensity and photoperiod of the light source [14–16], alkalinity and temperature of the cultivation broth [17], the diffuser type [18], liquid to biogas (L/G) ratio and gas-liquid flow configuration in the scrubbing column [19] on the quality of the biogas upgraded. These previous optimizations of the operational parameters allowed to obtain a biomethane complying with most international standards for its injection into natural gas networks. For instance, Franco-Morgado et al. [20] reported an average biomethane composition of 99.1% CH_4, 0.5% CO_2, 0.6% N_2 and 0.1% O_2 during the integral photosynthetic biogas upgrading in an analogous experimental set-up under indoors conditions. In addition, Marín et al. [21] obtained a CH_4 concentration between 85 and 98% in a pilot experimental set-up over one year operation under outdoor conditions. Rodero et al. [22] designed a control strategy based on the regulation of L/G ratio in order to maintain biomethane quality regardless of environmental fluctuations. In this study, a decrease in the pH of the cultivation medium mediated high liquid flowrates, with the subsequent increase in O_2 stripping and energy demand. In this context, Rodero et al. [17] reported an enhancement on CH_4 content in the upgraded biomethane from 79 to 98% with an increase on the inorganic carbon (IC) concentration in the cultivation medium from 100 to 1500 mg IC L^{-1}. Thereby, an optimum alkalinity capable of maintaining a high pH in the absorption column can support consistent CO_2 and H_2S removals. However, high IC concentrations in the pond could negatively impact on microalgae and bacterial activity due to a detrimental salinity effect, and increase CO_2 stripping from the cultivation medium to the atmosphere, thus limiting the environmental sustainability of photosynthetic biogas upgrading. For instance, de Farias Silva et al. [23] observed that the growth of *Synechococcus* PCC 7002 was inhibited at sodium bicarbonate concentrations above 22 g L^{-1} (~3140 mg IC L^{-1}) while Li et al. [24] reported a cell growth decrease from 120 to 1920 mg IC L^{-1} by addition of $NaHCO_3$ in *Chlorella vulgaris*. Besides, an inorganic salt content above 1-2 wt% might cause no salt-tolerant bacteria death due to cell plasmolysis [25]. Likewise, biomass concentration in the cultivation medium could potentially impact on both the CO_2 removal from biogas in the bubble column by promoting the accumulation of large algal-bacterial flocs in the vicinity of the biogas sparger, which could trigger biogas bubble coalescence and result in an inefficient CO_2

gas-liquid mass transfer [26], and its subsequent photosynthetic assimilation due to light limitation as a result of high biomass cell density.

This study systematically assessed the impact of long-term process operation under high IC concentration in the cultivation medium on the H_2S and CO_2 removal efficiency and robustness during photosynthetic biogas upgrading. Moreover, the influence of the biomass concentration on the performance of the upgrading process was also investigated. Finally, CO_2 stripping from the open pond was determined in order to evaluate the environmental performance of this technology.

2. Materials and methods

2.1. Experimental set-up

The experimental set-up, located indoors at the Institute of Sustainable Processes of Valladolid University (Spain), consisted of a High Rate Algal Pond (180 L) interconnected to a conical settler (8 L) whose supernatant was used as scrubbing solution in a 2.5 L absorption column and returned to the pond (Fig. 1). The pond (length: 202 cm , width: 63 cm, depth: 15 cm) was agitated by a 6-blade paddlewheel at a liquid recirculation velocity of ~20 cm s^{-1}, and illuminated continuously at 1240 ± 512 µmol m^{-2} s^{-1} (measured in different points along the total surface of the pond) by six Phillips LED PCBs (Spain). The pond (1.2 m^2 of illuminated surface) was continuously fed at an inlet flowrate of 3.2 L d^{-1} with a mineral salt medium (MSM) containing (g L^{-1}): 0.58 K_2HPO_4, 1.91 NH_4Cl, 0.10 $MgSO_4 \cdot 7H_2O$, 0.02 $CaCl_2 \cdot 2H_2O$, 5 mL of a trace metal solution (based on the *Spirulina* mineral salt medium [27]) and a mixture of $NaHCO_3$ and Na_2CO_3 according to the IC concentration set in during each operational stage at a pH of ~10. Synthetic biogas (70% CH_4, 29.5% CO_2 and 0.5% H_2S, Abello Linde (Spain)) was sparged into the scrubbing column (Ø: 4.4 cm, height: 165 cm) using a 2 µm metallic biogas diffuser at a flow rate of 50 ml min^{-1} and a recycling liquid to biogas ratio (L/G) of 0.5 according to Toledo-Cervantes et al. [28]. Despite counter-current flow operation involves higher CO_2 mass transfer rates, co-current mode was selected in this study since it entails lower O_2 and N_2 stripping which results in a higher biomethane quality. In addition, counter-current flow operation results in low dissolved O_2 concentrations in the liquid medium in the vicinity of the biogas sparger (at the bottom of the column), which induces the accumulation of elemental sulphur in the sparger and ultimately hinders CO_2 absorption [28]. Tap water was continuously added

to compensate evaporation losses from the open cultivation broth under operation with a zero effluent strategy.

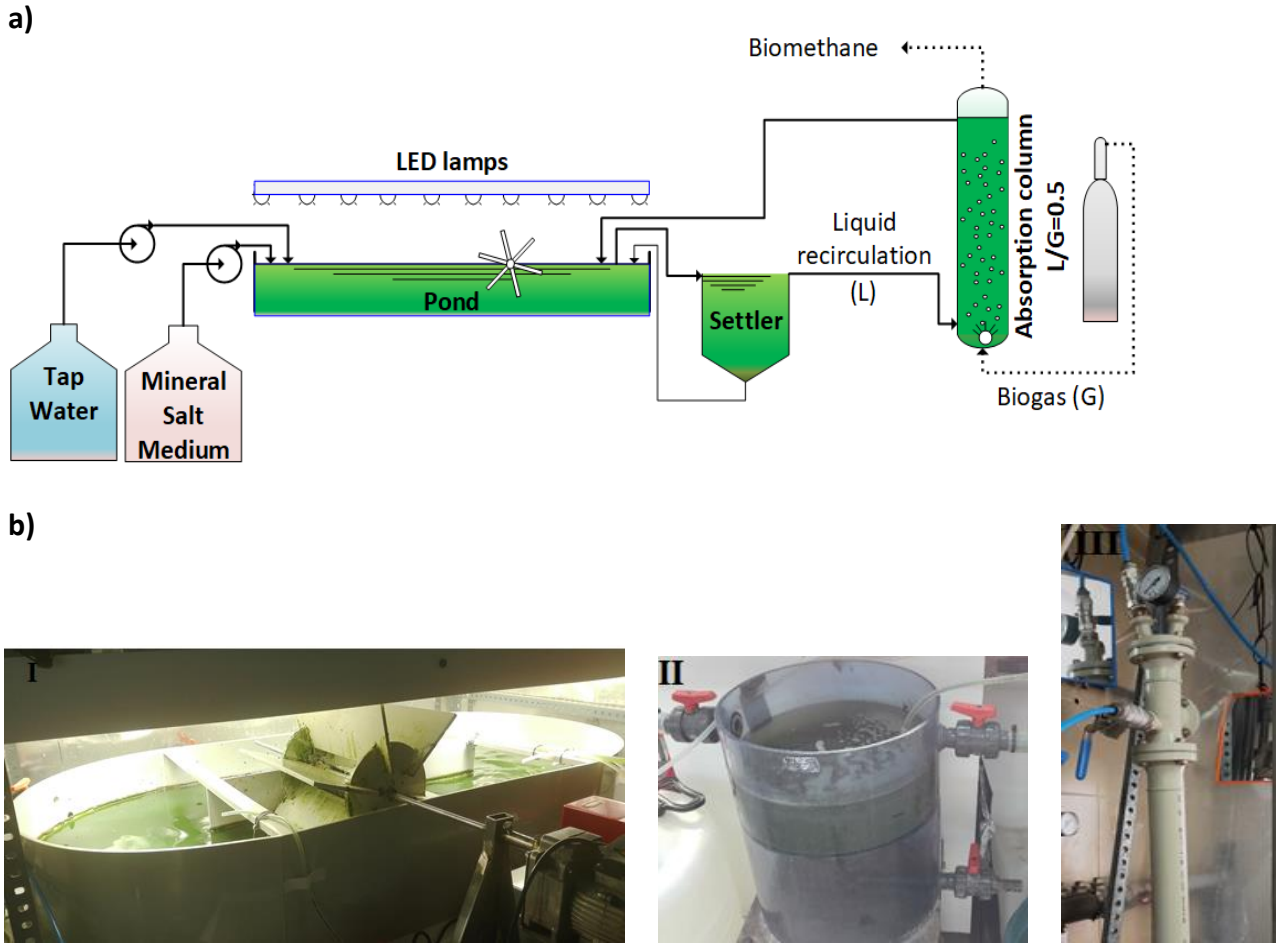

Fig. 1. a) Schematic diagram of the indoor experimental set-up for photosynthetic biogas upgrading and b) photograph of the pilot scale system: I pond, II settler, III biogas scrubbing column.

2.2. Operational conditions and sampling procedures

The pond was initially inoculated with a microalgal-bacterial consortium (previously acclimated to the MSM at 1200 mg IC L^{-1}) from an outdoors pond upgrading biogas at the Institute of Sustainable Processes. Three operational strategies were implemented to evaluate the influence of process operation under high alkalinity and biomass concentration in the pond (determined as volatile suspended solids, VSS) on the photosynthetic biogas upgrading efficiency and robustness (Table 1). During stage A, the pond was fed with MSM at an IC concentration of 1200 mg C L^{-1} and operated at a fixed biomass productivity of 15 g VSS m^{-2} d^{-1} set according to the nutrients fed to the pond and considering a phosphorous and nitrogen content in the microalgal biomass of 1 and 8%, respectively [19]. The algal-bacterial biomass was harvested in an external

tank via coagulation-flocculation with a synthetic polymeric flocculant derived from acrylamide (Chemifloc CV-300, Chemipol S.A.) followed by a sedimentation step. During stage B, the IC concentration of the MSM was increased to 2400 mg C L^{-1} and the IC concentration in the pond was adjusted accordingly by addition of $NaHCO_3/Na_2CO_3$ at the beginning of this operational stage. Biomass productivity at 15 g VSS m^{-2} d^{-1} was also maintained during stage B via coagulation-flocculation and sedimentation. In stage C, the operational conditions were similar to those in stage B but no algal-bacterial biomass was harvested.

Table 1. Operational conditions applied during the three operational stages.

Stage	**A**	**B**	**C**
Period (days)	0-65	66-113	114-134
Inorganic carbon in the feed (mg L^{-1})	1200	2400	2400
Productivity (g m^{-2} d^{-1})	15	15	-

Temperature, pH and dissolved oxygen (DO) concentration in the cultivation medium were daily monitored. The photosynthetic active radiation (PAR) was measured at the pond surface at the beginning of the study. Gas samples of 100 μL from the raw biogas and biomethane were drawn twice per week using gas tight syringes to determine the CH_4, CO_2, H_2S, O_2 and N_2 concentrations by GC-TCD. Biogas flowrates at the inlet and outlet of the scrubbing column were also measured to calculate CO_2 and H_2S removal efficiencies. Liquid samples of 100 mL from the MSM and the cultivation medium were drawn twice per week and filtered through 0.20 μm nylon filters to monitor dissolved TN, N-NH_4^+, N-NO_2^-, N-NO_3^- and IC. Aliquots of 50 mL were also drawn from the cultivation medium twice per week to monitor the VSS concentration. The flowrate of tap water was measured twice per week to determine evaporation losses. The maximum quantum yield of photosystem II (PSII) defined as the ratio of variable to maximal fluorescence (Fv/Fm) was measured at the end of stage C.

2.3. Determination of the mass transfer performance and CO_2 stripping rate

The gas-liquid mass transfer performance of the pond was assessed by means of respirometric measurements under controlled conditions, considering the O_2 transfer rate (OTR), O_2 production rate (OPR) and the O_2 uptake rate (OUR) according to the following mass balance under light conditions:

$$\frac{dC_L}{dt}(gO_2\ m^{-3}h^{-1}) = OTR(gO_2\ m^{-3}h^{-1}) + OPR(gO_2\ m^{-3}h^{-1}) - OUR(gO_2\ m^{-3}h^{-1}) \quad (1)$$

Defining the terms OTR, OPR and OUR, Equation 1 can be written as follows:

$$\frac{dC_L}{dt}(gO_2\ m^{-3}h^{-1}) = k_La_{O2}(h^{-1}) \cdot (C^*\text{-}C_L)(gO_2\ m^{-3}) + PO_2(gO_2\ gSSV^{-1}h^{-1}) \cdot X(gSSV\ m^{-3})\text{-}(R_{end} + R_{ex})(gO_2\ m^{-3}h^{-1}) \quad (2)$$

where k_La_{O2}, C^* and C_L are the volumetric oxygen mass transfer coefficient, the O_2 saturation concentration and the O_2 concentration at time *t* in the cultivation medium, respectively. PO_2 and X stand for the specific O_2 production and the biomass concentration, respectively. R_{end} and R_{ex} are the volumetric O_2 consumption rates due to endogenous biomass respiration and H_2S oxidation, respectively.

In the absence of air-liquid mass transfer and H_2S supply under illuminated conditions, Equation 2 can be written as follows:

$$\frac{dC_L}{dt}(gO_2\ m^{-3}h^{-1}) = PO_2(gO_2\ gSSV^{-1}h^{-1}) \cdot X(gSSV\ m^{-3})\text{-}R_{end}(gO_2\ m^{-3}h^{-1}) \quad (3)$$

On the other hand, in the absence of air-liquid mass transfer and H_2S supply under dark conditions, Equation 2 can be written as follows:

$$\frac{dC_L}{dt}(gO_2\ m^{-3}h^{-1}) = \text{-}R_{end}(gO_2\ m^{-3}h^{-1}) = \text{-}QO_2(gO_2\ gSSV^{-1}h^{-1}) \cdot X(gSSV\ m^{-3}) \quad (4)$$

where, QO_2 is the specific O_2 uptake rate.

The term R_{ex} can be estimated from the H_2S elimination capacity (EC) and the stoichiometric amount of O_2 required for the full oxidation of the absorbed H_2S into sulfate (1.9 g O_2 g $H_2S_{removed}^{-1}$):

$$R_{ex}(gO_2\ m^{-3}h^{-1}) = EC(gH_2S\ m^{-3}h^{-1})\frac{1.9gO_2}{gH_2S} \quad (5)$$

The experimental determination of QO_2 and PO_2 required to assess OUR and OPR, respectively, was carried out as follows: when the pond coupled with the biogas scrubbing column reached a stable H_2S removal, an aliquot from the cultivation medium of known biomass concentration was introduced into a 2.1 L glass bottle covered with aluminum foil to avoid photosynthetic activity and the temperature maintained by a water jacket at 28 ± 2°C. The test bottle was provided with magnetic stirring (300 rpm) and an optical dissolved O_2 sensor (Vernier, Oregon, USA) connected to a computer for data acquisition each 10 s. No headspace was allowed to avoid interfacial air-liquid mass transfer. Under these conditions, QO_2 was experimentally determined according to Equation 4 (QO_2 being the slope of the C_L vs time plot). The same experimental setup was used for PO_2 determination according to Equation 3, with PO_2 as the fitting

parameter. However, in this case the bottle was not covered with aluminum foil and provided with a similar PAR than that of the pond.

Once OPR and OUR were determined, dark conditions were applied to the pond coupled with the scrubbing column operating under steady conditions by turning off the LED lamps. The optical dissolved O_2 sensor placed in the pond measured the progressive depletion of O_2 under dark conditions. When dissolved O_2 concentration reached a minimum value of ~1 g m^{-3}, the LED lamps were turned on. Equation 2 was used to model dissolved O_2 data under illuminated conditions with k_La_{O2} as the fitting parameter. The volumetric CO_2 mass transfer coefficient (k_La_{CO2}) was then estimated from k_La_{O2} according to Estrada et al.[29]. In brief, the mass transfer coefficient through an aqueous layer for a given gas substrate can be predicted based on its molecular volume at the boiling point (V_m) as:

$$k_La \; \alpha \; \left(\frac{1}{V_m}\right)^{0.4} \tag{6}$$

Therefore, the mass transfer coefficient k_La_{CO2} can be estimated from a reference coefficient (k_La_{O2}) previously determined in the same reactor under identical operating conditions as follows:

$$\frac{k_La_{CO2}}{k_La_{O2}} = \frac{\left(\frac{1}{V_{m,CO2}}\right)^{0.4}}{\left(\frac{1}{V_{m,O2}}\right)^{0.4}} \tag{7}$$

V_m values of 34.0 and 25.6 mL mol^{-1} for CO_2 and O_2 were used [30]. A 4th-order Runge–Kutta method was used to solve Equations 2-4, while the Levenberg–Marquardt method was used for parameter fitting using ModelMaker^{TM} (Cherwell Scientific, UK).

2.4. Analytical methods

The pH was monitored using a pH meter Eutech Cyberscan pH 510 (Eutech instruments, The Netherlands), while an Oxi 330i oximeter (WTW, Germany) was used for DO and temperature determination in the cultivation medium of the pond. CO_2, H_2S, O_2, N_2 and CH_4 biogas and biomethane concentrations were determined using a Bruker 430 GC-TCD (Palo Alto, USA) equipped with the following columns: a CP-Pora BOND Q (25 m × 0.53 mm × 15 μm) and a CP-Molsieve 5A (15 m × 0.53 mm × 15 μm), with helium as the carrier gas at 18 psi. The detector, injector and oven temperatures were maintained at 200, 150 and 45 ºC, respectively. Dissolved IC and TN

concentrations were measured by means of a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 module. $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ concentrations were determined by HPLC-IC according to Serejo et al. [19]. $N\text{-}NH_4^+$ concentration was measured using a selective electrode Orion Dual Star (Thermo Scientific, The Netherlands) and VSS analyses were carried out according to standard methods [31]. PAR was determined with a LI-250A lightmeter (LI-COR, Germany). The maximum quantum yield of PSII was analyzed using an Aquapen-C fluorometer (Photon Systems Instruments, Czech Republic).

3. Results and discussion

3.1. Photobioreactor performance

The temperature of the cultivation medium in the pond remained almost constant at an average value of 28.2 ± 1.3 ºC, which resulted in an average evaporation rate of 6.9 ± 0.7 L m^{-2} d^{-1} along the three operational stages (Table 2). These water losses by evaporation were similar to those reported by Posadas et al. [32] in a similar outdoor pond during summer conditions. Similar pH values (9.7 ± 0.1) were observed in the three operational stages, supported by the high IC concentrations, which entailed a high buffer capacity of the cultivation medium [15]. On the other hand, the gradual increase in IC concentration exerted a negative impact on microalgal photosynthetic activity, as indicated by the gradual decrease in DO concentration in the cultivation medium. Average DO concentrations of 12.8 ± 1.9, 8.6 ± 0.9 and 4.4 ± 1.2 were measured during stages A, B and C, respectively (Table 2). The decrease in DO from stage A to B could be caused by oxidative stress in the cyanobacterial/microalgal culture induced by the increase of the salt content in the pond, which ultimately decreased photosynthetic activity [33]. During stage C, the decrease in DO concentration could be attributed to the lower photosynthetic activity as a result of the higher oxidative stress due to IC accumulation, and consequently, higher salinity in the pond, along with the lower light availability and the higher endogenous oxygen consumption by photorespiration at the higher biomass concentrations prevailing in stage C. In addition, the maximum photochemical quantum yield (Fv/Fm), which is an indicator of the photosynthetic performance of PSII since it determines the maximal conversion of light into chemical energy of PSII, was 0.28 at the end of the stage C. This value was lower than those typically reported for microalgae and cyanobacteria under no stress conditions (0.46-0.75) [34–36]. Low Fv/Fm indicates an impairment of PSII activity, which may be

caused by the inhibition of the activity of the PSII reaction centers or the electron transport at both sides of PSII (donor and acceptor) under stress conditions [37]. Despite the low DO levels recorded in the cultivation medium during stage C, those values were high enough (>2 mg O_2 L^{-1}) to support the aerobic bacterial activity responsible of nitrification and H_2S oxidation to SO_4^{2-} [38,39].

Table 2. Average environmental parameters (n=12) in the cultivation medium along with their corresponding standard deviation under steady state conditions during the three operational stages tested.

Stage	**A**	**B**	**C**
Cultivation broth temperature (ºC)	27.6 ± 0.6	29.5 ± 0.6	29.4 ± 0.6
DO (mg L^{-1})	12.8 ± 1.9	8.6 ± 0.9	4.4 ± 1.2
pH	9.7 ± 0.1	9.8 ± 0.1	9.7 ± 0
Evaporation rate (L m^{-2} d^{-1})	6.4±1.5	7.0 ± 0.6	6.8 ± 0.4

The initial concentration of VSS in the pond was 1.3 g L^{-1}, which decreased to steady state values of 0.8 ± 0.1 g L^{-1} during stage A (Fig. 2). The increase in the IC concentration during stage B led to a decrease in biomass concentration to steady state concentrations of 0.4 ± 0.1 g VSS L^{-1} (Fig. 2). VSS concentrations during stages A and B were determined by the biomass productivity actively maintained (15 g m^{-2} d^{-1}) and microalgal activity, which itself was influenced by the alkalinity in the pond. During stage C, no biomass was harvested, thus resulting in an increase in biomass concentration up to 1.38 g VSS L^{-1} by the end of stage C. However, biomass productivities (calculated as the increase of the mass of algal-bacterial biomass during a period of time and divided by the illuminated surface) of 13.3 g m^{-2} d^{-1} from day 114 to 126, and 3.4 g m^{-2} d^{-1} from day 126 onwards, were obtained during stage C, which represented a decrease in productivity compared to stages A and B (15 g m^{-2} d^{-1}). The lower biomass productivity by the end of stage C could be attributed to a higher oxidative stress of microalgae (mediated by the higher alkalinity), a decrease in light availability induced by the higher biomass concentration or the accumulation of inhibitory compounds in the cultivation medium under process operation without effluent and no biomass harvesting.

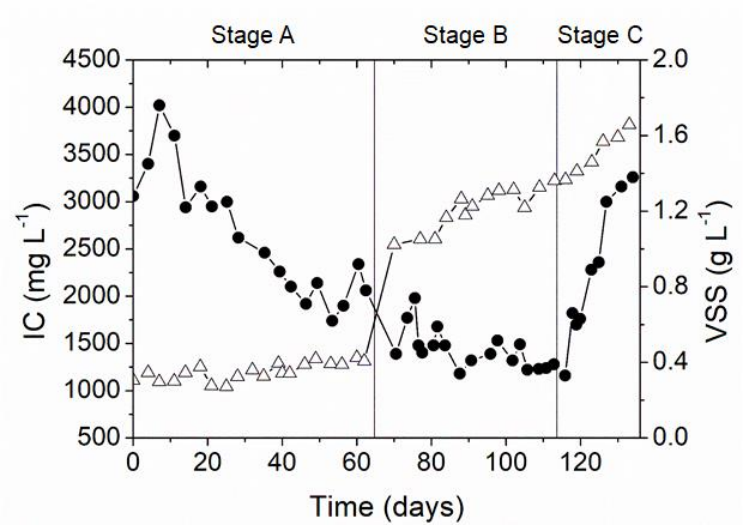

Fig. 2. Evolution of the concentration of inorganic carbon (IC, Δ) and volatile suspended solids (VSS, ●) in the pond.

IC concentration in the cultivation medium of the pond was adjusted at 1200 and 2400 mg C L^{-1} at the beginning of stages A and B, respectively. In stage A, the IC concentration in the pond remained almost constant at 1203 ± 93 mg C L^{-1}. However, the IC concentration in the cultivation medium increased during stages B and C along with the decrease in photosynthetic activity and triggered by the higher IC load in the MSM fed to the pond, reaching values of 3152 and 3814 mg C L^{-1} at the end of stages B and C, respectively (Fig. 2). In this context, Marín et al. [21] reported an increase in IC concentration up to 4138 mg L^{-1} using high-strength digestate (2000 mg IC L^{-1}) in a similar system located outdoors and operated with a zero effluent strategy. In addition, the high pH in the cultivation broth (9.7 ± 0.1) prevented a massive IC loss by CO_2 stripping as latter described in section 3.3.

Similar average TN concentrations in the pond were recorded under steady state in the three stages (609.1 ± 9.7, 558.5 ± 13.6 and 608.6 ± 16.2 mg N L^{-1} in stages A, B and C, respectively) (Fig. 3). Although N was added to the pond in form of ammoniacal species, no N-NH_4^+ was detected in the cultivation broth as a result of an active nitrification to NO_2^-/NO_3^- and NH_4^+ uptake by microorganisms. In fact, despite the high pH in the cultivation broth, the nitrogen mass balance conducted indicated that only 18, 13 and 1% of the initial nitrogen and the total nitrogen input was lost via volatilization during stages A, B and C, respectively (Table S1, Supplementary Material). Surprisingly, the predominant form of dissolved nitrogen during stages A and B was N-NO_2^- (average N-NO_2^- concentrations of 389.2 ± 5.6 and 404.3 ± 35.0 mg N L^{-1}, and

average N-NO_3^- concentrations of 226.7 ± 11.0 and 133.1 ± 31.2 mg N L^{-1} under steady state in stages A and B, respectively) despite the DO concentration in the pond remained always above saturation. This higher concentration of N-NO_2^- compared to N-NO_3^- could be explained by the higher growth rate of ammonia-oxidizing bacteria (AOB) compared to nitrite-oxidizing bacteria (NOB) at temperatures over 27 ºC, photoinhibition of NOB due to excessive light irradiance, a potential NOB activity inhibition due to high salinity and/or preferential N-NO_3^- assimilation by microalgae as a result of N-NH_4^+ depletion in the cultivation medium [38,40,41]. Interestingly, N-NO_3^- was the dominant specie of N during stage C despite the lower DO, with a final concentration of 540 mg N L^{-1} almost 10 folds higher than that of N-NO_2^- (55 mg N L^{-1}) (Fig. 3). These results could be attributed to the lower average irradiance in the cultivation medium due to a mutual shading effect caused by the increase in both biomass concentration and residence time, which likely enhanced NOB growth and nitrite oxidation. This high nitrate concentration could have contributed to microalgae inhibition during stage C since nitrate uptake rate is typically lower than that of ammonia and high nitrate concentration in the cultivation medium could cause an accumulation of intracellular nitrite [42].

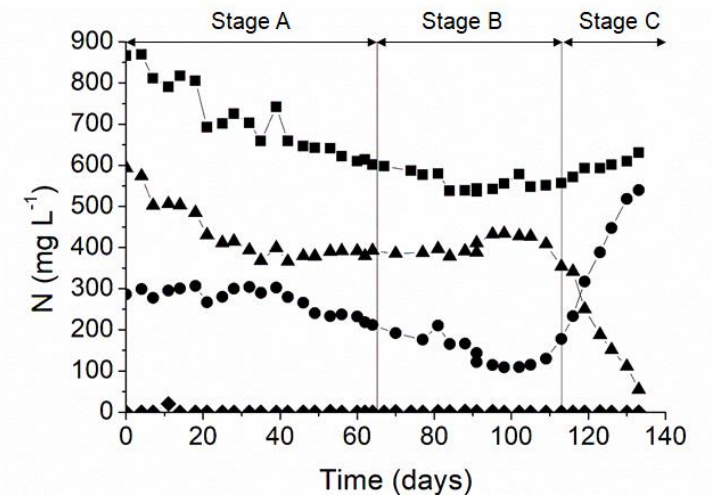

Fig. 3. Evolution of the concentration of nitrogen compounds in the pond: total nitrogen (■), N-NH_4^+ (♦), NO_2^- (▲) and NO_3^- (●).

3.2. Biogas upgrading

During stage A, the CO_2 concentration in the upgraded biogas varied from 1.5 to 4.4%, which corresponded to CO_2-REs between 96.6 and 89.5%, respectively. A more robust biogas upgrading was obtained as a result of the increase in IC concentration in stage B, where CO_2 concentrations ranged from 0.6 to 0.8% (corresponding to CO_2-REs ranging

between 98.4 and 98.1%). Similarly, CO_2 concentrations between 0.6 and 1.0% and CO_2-REs from 97.5 to 98.6% were recorded in stage C (Fig. 4a). These results were in agreement with Marín et al. [21], who reported CO_2 concentrations fluctuating between 2.6 and 11.9% in the upgraded biogas at IC concentrations of 1500-2000 mg C L^{-1}, which decreased to 0.7-2.1% at IC concentrations > 2800 mg C L^{-1}. Similarly, Rodero et al. [43] observed a CO_2 concentration increase from 2.7 to 12% due to the decrease in the pH of the cultivation medium from 9.50 to 9.05 at an IC concentration of ∼1900 mg C L^{-1}. In this particular study, the increase in the alkalinity of the cultivation medium from 1200 to 2400 mg IC L^{-1} supported stable CO_2 concentrations in the upgraded biogas and improved the robustness of the upgrading process. These low CO_2 levels complied with the most restrictive values according to the recent European standard EN 16723-1 for biogas injection into natural gas networks (≤ 2%) [4]. On the other hand, the CO_2 values recorded during stage C gradually increased along with the increase in the algal-bacterial biomass (Fig. 2 and 4). The high biomass concentrations prevailing at the end of stage C could have negatively impacted on the CO_2 gas-liquid mass transfer in the scrubbing biogas column as a result of biomass build-up on the diffuser. However, this effect of the biomass concentration on CO_2 removal was no significant ($p>0.05$, one-way ANOVA) due to the high IC concentration in the cultivation medium (up to 3814 mg C L^{-1} by the end of stage C).

On the other hand, H_2S-REs of 100% were achieved regardless of the alkalinity (1100-3800 mg IC L^{-1}) and the biomass concentration (0.3-1.38 g SSV L^{-1}) in the cultivation medium. These higher eliminations compared to CO_2-REs were mediated by the higher aqueous solubility of H_2S relative CO_2 according to their dimensionless Henry's law constants (C_L/C_G, $H_{H2S} \approx 2.44$ *vs* $H_{CO2} \approx 0.83$ at 25 °C) and the rapid oxidation of H_2S in the liquid phase [44,45]. In this context, the high DO concentration and pH typically encountered in algal-bacterial ponds lead to the formation of SO_4^{2-} as the major end-product of H_2S oxidation which can be chemically supported by the DO concentration in the cultivation medium and/or biologically by the action of aerobic sulfur-oxidizing bacteria, i.e. Thioalbus genus [43,46]. Similarly, a complete H_2S removal was obtained regardless of the environmental conditions variations in a similar system over one year operation using a high alkalinity digestate [21]. Franco-Morgado et al. [15] also reported H_2S-REs of 99.5 ± 0.5% during biogas upgrading at IC concentrations in the

cultivation medium > 1000 mg C L^{-1}. These results confirmed the long-term robustness of algal-bacterial processes under high-alkalinity conditions for H_2S removal.

The low L/G ratio implemented in this study (0.5) constrained the amount of N_2 and O_2 stripped out from the recycling liquid to the biogas in the scrubbing column. In this regard, average N_2 concentrations of 1.3 ± 0.4, 1.0 ± 0.3 and 0.8 ± 0.3%, and O_2 concentrations of 0.2 ± 0.1, 0.1 ± 0.1 and 0 ± 0.1% were recorded in the upgraded biogas during stage A, B and C, respectively (Fig. 4c). Although a slight decrease in N_2 and O_2 desorption was recorded during stages B and C, these differences were minimal. In fact, no-correlation between the alkalinity and N_2 and O_2 stripping was obtained in a similar experimental set-up at IC concentrations ranging from 100 to 1500 mg C L^{-1} at an L/G ratio of 0.5 [17]. The O_2 content in the upgraded biogas along the three stages was below the regulatory limits for biomethane injection into natural gas networks or its use as vehicle fuel (≤ 1%) as a result of the low L/G ratio set in this study.

Finally, CH_4 concentrations in the biomethane ranged from minimum values of 94.6, 97.8 and 98.0% to maximum values of 97.5, 98.9 and 98.7%, during stages A, B and C, respectively (Fig. 4b). Although, a good biomethane quality in terms of CH_4 concentration (≥ 95%) was achieved in the three operational stages, these values were more stable during stages B and C as a result of the consistent CO_2 removal and the low O_2 and N_2 stripping. In this context, the CH_4 concentrations achieved in this study were comparable to those recently reported in outdoors systems. Thus, Rodero et al. [43] recorded a CH_4 concentration of 97.3% in a similar configuration system at semi-industrial scale operating at a L/G ratio of 0.8, pH 9.5 and an IC concentration in the pond of ∼1900 mg C L^{-1}, while Marín et al. [21] obtained a maximum CH_4 concentration of 97.8% in the upgraded biogas operating at a L/G ratio of 1, IC concentrations in the cultivation medium >2780 mg C L^{-1} and a pH of ~9.6.

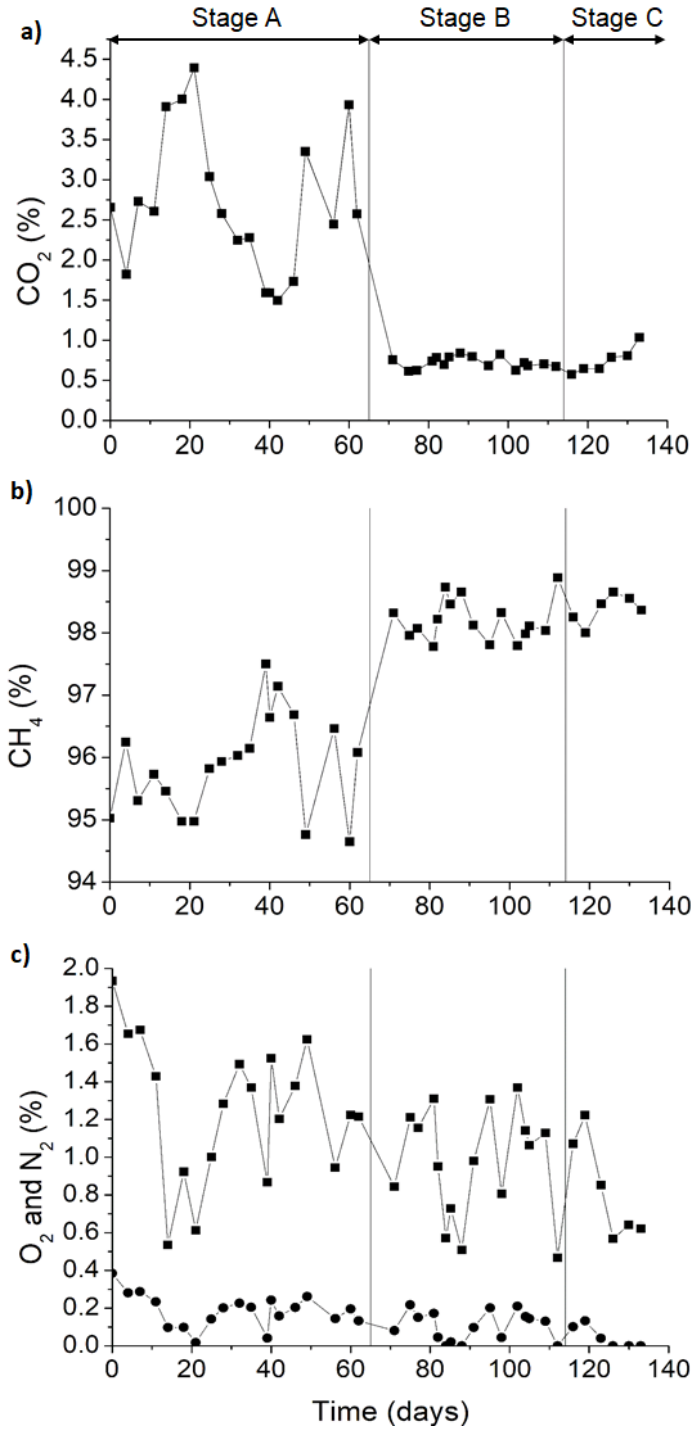

Fig. 4. Evolution of the concentration of a) CO_2, b) CH_4, c) O_2 (●) and N_2 (■) in the upgraded biogas.

3.3. Volumetric gas-liquid mass transfer coefficient and CO_2 stripping

The gas-liquid mass transfer performance of the open pond was evaluated under steady H_2S removal in stage B. The respirometric characterization performed in these days yielded average QO_2 and PO_2 values of 10.1 ± 3.0 and 11.3 ± 0.1 mg O_2 g SSV^{-1} h^{-1}, respectively. These QO_2 values were in agreement with previous studies reporting endogenous respiration rates of microalgae-bacteria cultures in the range of 4-6 mg O_2 g

VSS h^{-1} [47,48]. Likewise, Sforza et al. [49] reported PO_2 values in the range of 6-15 mg O_2 g VSS h^{-1} for microalgae-bacteria systems. The H_2S elimination capacity supported by the system was 107 mg H_2S m_{liquid}^{-3} h^{-1}, corresponding to a R_{ex} value of 204 mg O_2 m_{liquid}^{-3} h^{-1}. The values of QO_2, PO_2 and R_{ex} experimentally determined were used in Equation 2 to estimate k_La_{O2} and then k_La_{CO2} (Equation 7). The fitting of Equation 2 to the experimental dissolved O_2 concentrations is shown in Figure S1 (supplementary material). Correlation coefficients (R^2) ranging from 0.97 to 0.99 were obtained, which confirmed that the experimental data were adequately described by the model.

Considering the three mass transfer characterizations performed in the pond, average k_La_{O2} and k_La_{CO2} values of 1.18 ± 0.30 and 1.05 ± 0.27 h^{-1} were retrieved, respectively. The k_La_{O2} obtained in this study was in the range of that reported by Franco-Morgado et al. [15] (0.83 h^{-1}) in a 25 L pond with a depth of 14 cm and an internal recirculation velocity of 15 cm s^{-1}. Similarly, Ouargui et al. [50] reported a k_La_{O2} of 0.76±0.12 h^{-1} in a full-scale pond of 400 m long, 2.5 m uniform width and 0.5 m deep with a recirculation time of 79 min. In addition, Pham et al. [51] obtained k_La_{O2} values of 0.8-3.1 h^{-1} with a liquid recirculation velocity in the range of ~15-45 cm s^{-1} in a pond of 386 cm long × 40 cm wide × 15 cm deep. Based on the empirical IC concentration and pH value, the H_2CO_3 (dissolved CO_2) concentration was calculated considering the dissociation equilibria of the inorganic carbon (pKd_1 and pKd_2 of 6.35 and 10.33, respectively). CO_2 stripping was then estimated based on k_La_{CO2} and the dissolved CO_2 concentration in the pond under steady state in each operational stage. An average stripping rate of 0.43 ± 0.08, 0.94 ± 0.31 and 1.30 ± 0.09 g C-CO_2 m_{liquid}^{-3} h^{-1} was estimated during stages A, B and C, respectively, which showed that even at the high pH values recorded in the pond, CO_2 can be stripped out due to the high IC concentration. These values corresponded to 14.5, 24.1 and 33.4% of the IC input to the system (C-CO_2 absorbed from the biogas and IC added in the MSM) in stages A, B and C, respectively. In this context, Meier et al. [14] recorded higher IC losses to the atmosphere of 57% in an open-photobioreactor at a cultivation broth pH of ~7.3. Based on IC equilibrium, the CO_2 stripping potential increases exponentially as pH decreases. However, these results were higher than the 5% reported by Toro-Huertas et al. [52] in an alkaline cultivation medium (IC concentration of 1320±140 mg IC L^{-1}) in a high rate algal pond operated at a recirculation velocity of ~ 15 cm s^{-1} and a pH values between 9.3 and 9.8.

4. Conclusions

The alkalinity in the cultivation medium impacted both on the efficiency of CO_2 removal in the biogas scrubbing column and on CO_2 fixation by microalgae in the pond. IC concentrations > 2400 mg C L^{-1} enhanced the effectiveness and robustness of the upgrading process at the expenses of a decreasing photosynthetic activity due to oxidative stress of microalgae. In addition, high alkalinities can mediate high CO_2 stripping even at high pH values, thereby decreasing the environmental benefits of this green technology. Finally, an increase in biomass concentration induced a slight decrease on the CO_2 gas-liquid mass transfer in the biogas scrubbing column and lower biomass productivities in the pond.

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Supplementary Material

Long-term influence of high alkalinity on the performance of photosynthetic biogas upgrading

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Content:

Table S1. Nitrogen mass balance during the three operational stages tested.

STAGE	N initial (g)	N inlet MSM (g)	N biomass (g)	N final (g)	N volatilized (g)
A	164.5	104.0	94.4	125.4	48.8
B	125.4	76.8	69.7	105.8	26.7
C-I*	105.8	19.2	15.4	112.6	0
C-II*	112.6	12.8	3.9	119.7	1.8

Where:

Initial N was the total concentration of dissolved nitrogen in the culture broth at the beginning of each stage multiplied by the total volume.

N inlet MSM was the total amount of nitrogen added as $N\text{-}NH_4^+$ in the mineral salt medium (MSM) during each stage calculated as: N concentration in the MSM (g L^{-1}) × flowrate inlet MSM (L d^{-1}) × duration stage (d)

N biomass was the nitrogen assimilated into microalgae biomass considering a nitrogen content in the microalgal biomass of 8% calculated as: productivity of biomass (g SSV m^{-2} d^{-1}) × N content biomass (0.08 g N g^{-1} SSV) × surface illuminated (m^2) × duration stage (d).

Final N was the total concentration of dissolved nitrogen in the culture broth at the end of each stage multiplied by the total volume.

N volatilized as $N\text{-}NH_4^+$ was estimated from the mass balance as:

Initial N + N inlet MSM - N biomass - Final N

*Stage C was divided into two parts since the biomass productivity was different along this period.

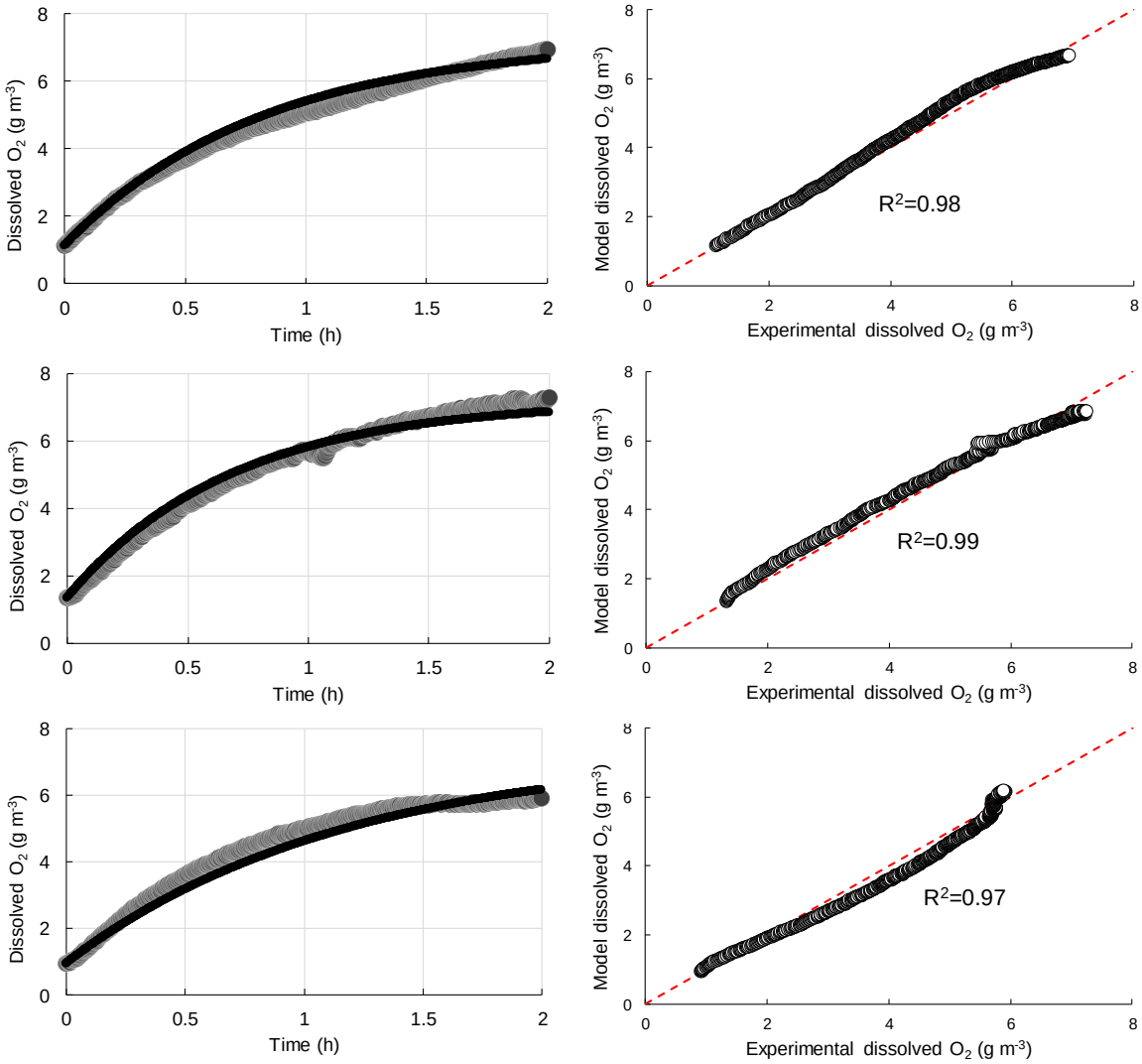

Fig. S1. Fitting of Equation 2 (solid black line) to the experimental dissolved O_2 data (circles) measured in the pond coupled to the absorption unit and the corresponding correlation curve.

Chapter 5

Technology validation of photosynthetic biogas upgrading in a semi-industrial scale algal-bacterial photobioreactor

Rodero, M.del R., Lebrero, R., Serrano, E., Lara, E., Arbib, Z., García-Encina, P.A., Muñoz, R., 2019. Bioresour. Technol. 279, 43–49. doi:10.1016/j.biortech.2019.01.110

Technology validation of photosynthetic biogas upgrading in a semi-industrial scale algal-bacterial photobioreactor

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ABSTRACT

The performance of photosynthetic biogas upgrading coupled to wastewater treatment was evaluated in an outdoors high rate algal pond (HRAP) interconnected to an absorption column at semi-industrial scale. The influence of biogas flowrate (274, 370 and 459 L h^{-1}), liquid to biogas ratio (L/G = 1.2, 2.1 and 3.5), type of wastewater (domestic *versus* centrate) and hydraulic retention time in the HRAP (HRT) on the quality of the biomethane produced was assessed. The highest CO_2 and H_2S removal efficiencies (REs) were recorded at the largest L/G due to the higher biogas-liquid mass transfer at increasing liquid flowrates. No significant influence of the biogas flowrate on process performance was observed, while the type of wastewater was identified as a key operational parameter. CO_2 and H_2S-REs of 99% and 100% at a L/G_{max}=3.5 were recorded using centrate. The maximum CH_4 content in the biomethane (90%) was limited by N_2 and O_2 desorption.

Keywords: algal-bacterial photobioreactor; biogas upgrading; microalgae; semi-industrial scale HRAP; wastewater treatment.

1. Introduction

Biogas from the anaerobic digestion of organic waste, such as sludge from wastewater treatment plants (WWTPs), constitutes a valuable bioenergy vector able to reduce our current dependence on fossil fuels. Biogas from WWTPs is typically composed of CH_4 (60-75%), CO_2 (30-40%) and other pollutants at trace level concentrations such as H_2S (0.02-2%), O_2 (0-1%), N_2 (0-2%), NH_3 (<1%) and siloxanes (0-0.2%) (Ryckebosch et al., 2011). The high concentration of CO_2 increases hydrocarbon and carbon monoxide emissions during biogas combustion, reduces its specific calorific value and increases its transportation cost. On the other hand, H_2S is a malodorous and toxic gas contaminant that generates corrosion and mechanical wear in pipelines and internal combustion engines (Lebrero et al., 2016).

Several technologies are nowadays commercially available to remove these contaminants from biogas in order to generate a high quality biomethane similar to natural gas. Physical-chemical technologies for CO_2 separation such as pressure swing adsorption, membrane separation and water/organic/chemical scrubbing often need a previous H_2S cleaning step (i.e. adsorption on activated carbon or metal ions-based *in situ* precipitation) and a high energy input (0.2-0.7 kWh/m^3_{biogas}), with the associated increase in operational costs. Thus, the high energy and chemical requirements of conventional biogas upgrading processes, among other factors such as the cost of acquisition of the organic substrate and the type of digestion process, limit the cost-effective use of biomethane as a renewable substitute of natural gas (Rodero et al., 2018a). On the other hand, biological technologies such as biofiltration or *in situ* microaerobic anaerobic digestion for H_2S removal followed by hydrogenotrophic biogas upgrading (*power to gas*) for CO_2 bioconversion into CH_4 entail the need of a two-stage process and can be only applied in locations with a sustained surplus of renewable electricity (Angelidaki et al., 2018; Muñoz et al., 2015a).

In this context, biogas upgrading using algal-bacterial processes has emerged as a cost-competitive and environmentally friendly platform capable of removing CO_2 and H_2S in a single step process (Bahr et al., 2014). Photosynthetic biogas upgrading is based on the concomitant CO_2 fixation by microalgae using solar energy and oxidation of H_2S to S^0/SO_4^{2-} by sulfur-oxidizing bacteria using the oxygen photosynthetically produced (Sun et al., 2016). Moreover, this biotechnology simultaneously supports wastewater

treatment since residual nutrients can sustain algal-bacterial growth, which contributes to improve its environmental and economic sustainability (Posadas et al., 2015a; Zhang et al., 2017). Biogas upgrading combined with wastewater treatment in algal-bacterial photobioreactors has been successfully validated indoors at lab-pilot scale (Bahr et al., 2014; Meier et al., 2017; Ouyang et al., 2015; Posadas et al., 2016; Rodero et al., 2018b; Serejo et al., 2015; Toledo-Cervantes et al., 2017a, 2016; Yan et al., 2016). Likewise, promising results in terms of biogas upgrading (CH_4 contents of 85.2-97.9%) and centrate treatment (total nitrogen removal efficiencies (REs) of 80-87% and P-PO_4^{3-} REs of 85-92%) were obtained in an outdoors 180 L high rate algal pond (HRAP) interconnected to an absorption column (Marín et al., 2018; Posadas et al., 2017a). However, this innovative biogas upgrading technology has not been yet validated at semi-industrial scale, which is a must in order to foster its acceptance by the industrial sector.

This work investigated for the first time the influence of biogas flow rate and the liquid to biogas ratio (L/G) on biomethane quality in an outdoors algal-bacterial photobioreactor treating real biogas at semi-industrial scale. Moreover, the influence of the type of wastewater (domestic *versus* centrate) and the hydraulic retention time (HRT) in the HRAP on biogas upgrading and nutrient recovery efficiency was also assessed.

2. Materials and methods

2.1. Biogas and wastewaters

Biogas was produced in a semi-industrial 20 m^3 anaerobic digester treating sewage sludge at Chiclana de la Frontera WWTP (Spain). Biogas composition averaged 69.2±5.7% CH_4, 32.7±2.8% CO_2 and 1183±1006 ppm H_2S. Fresh domestic wastewater was pumped into the HRAP directly after screening and degreasing of the influent raw wastewater. The average composition of the domestic wastewater was (mg L^{-1}): chemical oxygen demand (COD) = 496±145, inorganic carbon (IC) = 46±11, total nitrogen (TN) = 41±11, ammonium (N-NH_4^+) = 44±9, phosphate (P-PO_4^{3-}) = 6±2 and total suspended solids (TSS) = 140±40. Urea, H_3PO_4, $NaHCO_3$ and Na_2CO_3 were added to the raw domestic wastewater to achieve a final IC, TN and P-PO_4^{3-} concentration of 500, 500 and 75 mg L^{-1}, respectively, in order to simulate a medium-strength centrate composition.

2.2. Experimental set-up

The experimental set-up was located outdoors at Chiclana de la Frontera WWTP (36.42 N; 6.15 W) (Spain). The set-up consisted of a 9.6 m^3 HRAP made of concrete blocks with an illuminated surface of 32 m^2, 0.3 m of depth, two water channels divided by a central wall and two flow rectifiers in each side of the curvature. The cultivation broth in the HRAP was continuously agitated by a 6-blade paddlewheel operated at 7 rpm, resulting in an internal liquid velocity of 0.30 m s^{-1}. The HRAP was interconnected to a 150 L absorption column provided with a polypropylene fine bubble biogas diffuser (Ecotec AFD 270) via an external liquid recirculation of the supernatant from a 7 m^3 conical settler (Figure 1). The algal-bacterial biomass accumulated at the bottom of the settler was continuously recirculated to the HRAP to avoid an excessive biomass accumulation in the settler. The algal-bacterial biomass was wasted from an overflow located in the HRAP in order to maintain the depth of the photobioreactor at 0.3 m.

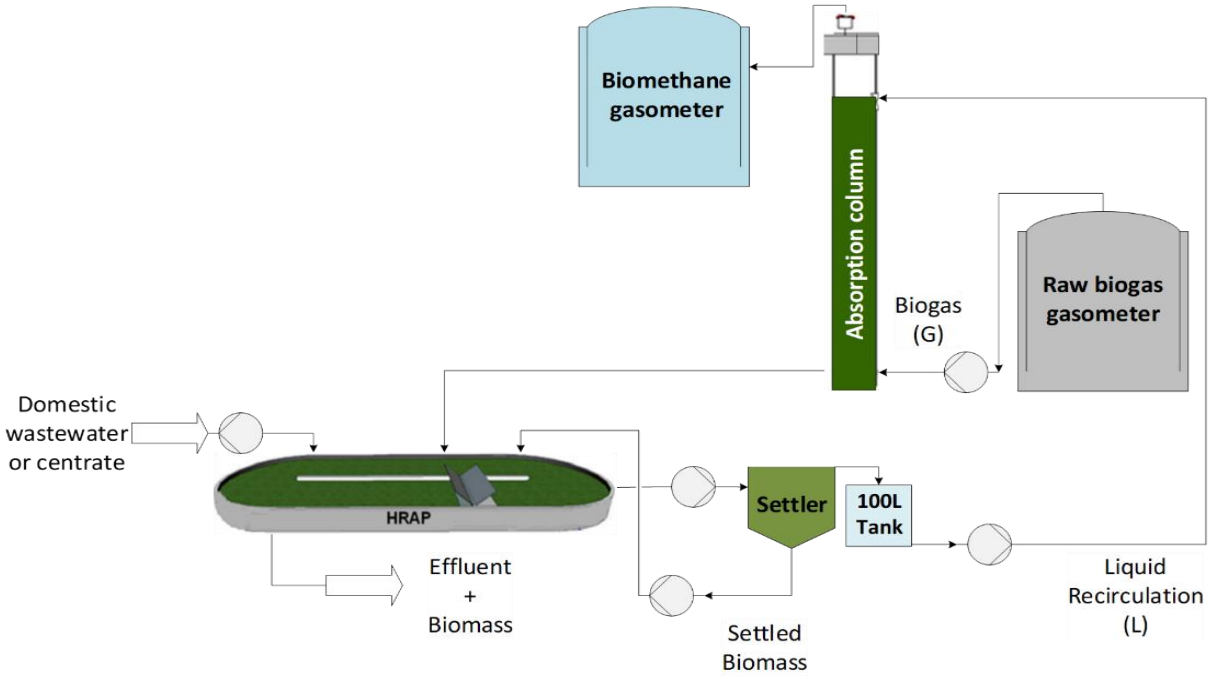

Figure 1. Schematic diagram of the experimental set-up.

2.3. Operational conditions and sampling procedures

The HRAP was inoculated with a consortium of cyanobacteria/microalgae and bacteria from an outdoors HRAP treating domestic wastewater at Chiclana de la Frontera WWTP prior to the experiment start-up. Three different operational conditions were

tested to assess the influence of the HRT and the type of wastewater used as a nutrient source (domestic wastewater *vs* centrate) in the HRAP on biogas upgrading efficiency. During stages I and II, the HRAP was fed with domestic wastewater at a HRT of 3.5 and 8 days, respectively, which correspond to typical values used during wastewater treatment in HRAPs (Arbib et al., 2013; Posadas et al., 2015b). In stage III, simulated centrate was used as a nutrient source at a high HRT (≈73 days) in order to avoid inhibition of microalgae growth by its high NH_4^+ concentration. The high nutrient content of centrate entailed lower wastewater flowrates to satisfy nutrient requirements. L/G ratios of 1.2 and 2.1 were tested under counter-current flow operation at different biogas flowrates (274±12, 370±7 and 459±36 L h^{-1}) under steady state in the three operational stages. Moreover, a L/G ratio of 3.5 was tested only at the lowest biogas flow rate of 274 L h^{-1} since the maximum flow rate of the recycling liquid pump was 1000 L h^{-1}.

The temperature, dissolved oxygen concentration (DO) and pH in the cultivation broth of the HRAP were monitored every five minutes. Liquid samples of 1 L from the influent wastewater (obtained along 24 hours) and 500 mL from the clarified effluent were withdrawn twice a week to monitor the concentration of COD, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, IC and TN. Liquid samples were also drawn from the cultivation broth of the HRAP to monitor algal-bacterial TSS and volatile suspended solids (VSS) concentration. The algal-bacterial biomass was dried for 24 h at 105 °C to determine its elemental composition (C, N and S) under steady state in each operational stage.

2.4. Analytical procedures

The pH, DO concentration and temperature were monitored and recorded using Crison pH 4603 and DO 6050 probes coupled to a Crison Multimeter 44 display (Spain). CH_4, CO_2, H_2S and O_2 were measured using a COMBIMASS® Portable Gas-analyzer GA-m5. The concentrations of dissolved TN and IC were determined by means of a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. NH_4^+ was analyzed using a selective electrode (Thermo Scientific Orion, USA). COD, $P\text{-}PO_4^{3-}$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, TSS and VSS were measured using Standard Methods (Eaton et al., 2005). The elemental composition of the algal-bacterial biomass (C, N and S content) was determined using a LECO CHNS-932 analyzer (LECO, Italy).

2.5. Statistical analysis

The results here presented were provided as the average values along with their standard deviation from replicate measurements. An analysis of variance (ANOVA) was performed to determine the influence of the biogas flowrate, HRT and L/G ratio on the quality of biomethane.

3. Results and discussion

3.1. Environmental parameters

The ambient temperature and the diurnal solar radiation cycle seasonally varied along the three experimental stages, with the subsequent variations in the cultivation broth temperatures (23.5±2.5, 12.4±2.3 and 18.8±3.0 ºC during stages I, II and III, respectively) (Table 1). These variations in environmental conditions are inherent to any outdoors experimentation. In this context, Rodero et al. (2018b) found a negligible impact of the temperature on biogas upgrading performance when using a moderate alkalinity cultivation broth (i.e. centrate), while at low alkalinity (i.e. domestic wastewater) the CH_4 content of the biomethane increased by 3.3% when the temperature decreased from 35 ºC to 12 ºC. The average pH of the cultivation broth under steady state during stages I, II and III was 7.3±0.2, 7.1±0.5 and 8.9±0.3, respectively. The higher pH recorded in the latter stage was attributed to the higher pH and alkalinity of the centrate fed to the HRAP in comparison with the domestic wastewater used during stages I and II. The maximum DO concentrations in the cultivation broth (8.3±2.8, 6.6±1.3 and 9.4±1.4 mg L^{-1} in stages I, II and III, respectively) (Table 1) were recorded during the daytime, and never exceeded inhibitory levels for microalgae activity (<25 mg O_2 L^{-1}) (Jiménez et al., 2003). On the other hand, minimum daily DO concentrations of 0.3±0.2, 2.8±1.4 and 4.3±0.7 were recorded in stages I, II and III, respectively, during the nighttime due to absence of photosynthetic activity and the occurrence of an active organic matter oxidation and NH_4^+ nitrification (Posadas et al., 2013). It is worth noticing that the lowest DO concentration was observed during the treatment of domestic wastewater at a HRT of 3.5 days due to the higher biological oxygen consumption resulting from the higher organic loading rates mediated by the shorter HRT (Arbib et al., 2017).

Finally, the average water losses by evaporation during stages I, II and III accounted for 14.7±18.7, 4.3±3.2 and -0.1±0.6 L m^{-2} d^{-1} (Table 1). The highest evaporation rate herein

recorded was ~ 2.2 times higher than the maximum values reported by Marín et al. (2018) in a 180 L outdoors HRAP located at Valladolid (Spain) during one year operation. This high value was attributed to the higher temperatures of the cultivation broth and the high turbulence at the HRAP surface caused by the wind in Chiclana de la Frontera. On the other hand, the negative value obtained during stage III was caused by the higher average rain recorded (4.4 L m^{-2} d^{-1}) during steady state in this period compared to 1.0 L m^{-2} d^{-1} recorded during state II and the absence of rain during stage I. This value agreed with the observations of Posadas et al. (2014), who reported negative evaporation rates in an outdoors HRAP.

Table 2. Average environmental parameters in the HRAP during the three operational stages tested under steady state conditions.

Parameter	Stage		
	I	II	III
Average ambient temperature (°C)	25.3±1.3	12.3±2.0	15.3±2.0
Average cultivation broth temperature (°C)	23.5±2.5	12.4±2.3	18.8±3.0
Average pH	7.3±0.2	7.1±0.5	8.9±0.3
Average maximum daily DO (mg O_2 L^{-1})	8.3±2.8	6.6±1.3	9.4±1.4
Average minimum daily DO (mg O_2 L^{-1})	0.3±0.2	2.8±1.4	4.3±0.7
Average evaporation rate (L m^{-2} d^{-1})	14.7±18.7	4.3±3.2	-0.1±0.6

3.2. Biogas upgrading performance

3.2.1. CO_2 removal

CO_2 removal efficiency was a function of the gas-liquid mass transfer in the absorption column, which itself was influenced by CO_2 consumption by microalgae in the HRAP. During stage I, CO_2-REs of 59.2±3.2, 76.6±1.8 and 88.9±1.5%, which corresponded to CO_2 concentrations of 17.3±2.2, 11.8±1.4 and 5.8±1.0% in the upgraded biogas, were recorded at L/G ratios of 1.2, 2.1 and 3.5, respectively, at a biogas flowrate of 274 L h^{-1}. CO_2-REs increased with the L/G ratio due to the increase in the overall gas-liquid mass transfer coefficient and the lower CO_2 transferred per volume of recirculating medium, which prevented the acidification of the recycling cultivation broth along the absorption column as a result of the acidic nature of biogas (Anbalagan et al., 2017; Posadas et al., 2017a). Indeed, a lower decrease in pH between the top and the bottom of the absorption column was observed with the increase in the L/G ratio (ΔpH of 1.7, 1.5 and 1.2 at a L/G ratio of 1.2, 2.1 and 3.5, respectively) during stage I. Similarly, CO_2-REs varied from 59.6±2.5 to 74.2±0.5% and from 64.4±2.2 to 81.0±0.3% when the L/G

increased from 1.2 to 2.1 at a biogas flowrate of 370 and 459 L/h, respectively (Figure 2a). In this context, a slight increase in CO_2-RE was recorded at the highest biogas flowrate as a result of the higher turbulence in the absorption column, which enhanced the gas-liquid mass transfer coefficient in this unit.

During stage II, CO_2-REs of 56.4±2.5, 77.2±1.5 and 90.4±0.4% were recorded at a L/G ratio of 1.2, 2.1 and 3.5, respectively, and a biogas flowrate of 274 L h^{-1} (Figure 2a). No significant differences ($p > 0.05$) were observed in CO_2-RE values compared to stage I, which revealed a negligible influence of the HRT on CO_2 removal efficiency when domestic wastewater was used to support algal-bacterial growth. In fact, although higher pH values were expected at longer HRTs based on the lower acidification caused by the reduction in CO_2 production due to the lower organic matter load, a similar pH of the cultivation broth was recorded in the HRAP in both stages as a result of the higher nitrifying activity during stage II (as discussed in section 3.3) (de Godos et al., 2016; Posadas et al., 2017b). The decrease in pH along the absorption column in stage II was similar to that recorded in stage I (ΔpH of 2.1, 1.7 and 1.5 at a L/G ratio of 1.2, 2.1 and 3.5, respectively), which was attributed to the similar IC concentration of the cultivation broth in both stages (25.6±5.5 and 29.5±9.4 mg L^{-1} during stage I and II, respectively, under steady state conditions). Similarly, CO_2-REs varied from 64.3±4.7 to 84.0±1.4% and from 63.6±0.4 to 80.1±0.4% when the L/G increased from 1.2 to 2.1 at biogas flowrates of 370 and 459 L h^{-1}, respectively. These results were in accordance to Anbalagan et al. (2017), who observed an increase in CO_2-RE from 45 to 79% when increasing the L/G ratio from 1 to 15 regardless the HRT.

Similarly, the lowest CO_2-REs during stage III were obtained at a L/G ratio of 1.2 (78.0±12.1, 85.3±1.3 and 77.6±1.0%, which corresponded to CO_2 concentrations of 10.1±4.4, 7.2±1.0 and 11.1±1.1 % in the upgraded biogas at 274, 370 and 459 L h^{-1}, respectively) (Figure 2a). An increase in CO_2-REs up to 97.8±0.8, 98.4±1.4 and 97.3±0.5% at 274, 370 and 459 L h^{-1}, respectively, was obtained at a L/G ratio of 2.1. Finally, the highest CO_2-REs (99.1±0.3%) were recorded at a L/G ratio of 3.5 (Figure 2a). The superior CO_2-REs obtained during this stage compared to stages I and II was likely due to the higher pH and alkalinity of the cultivation broth, which ultimately increased CO_2 and H_2S mass transfer in the absorption column as a result of the lower decreases in pH (ΔpH of 1.9, 1.3 and 0.8 at a L/G ratio of 1.2, 2.1 and 3.5, respectively, in the assays conducted at a biogas flowrate of 274 L h^{-1} of biogas flowrate).

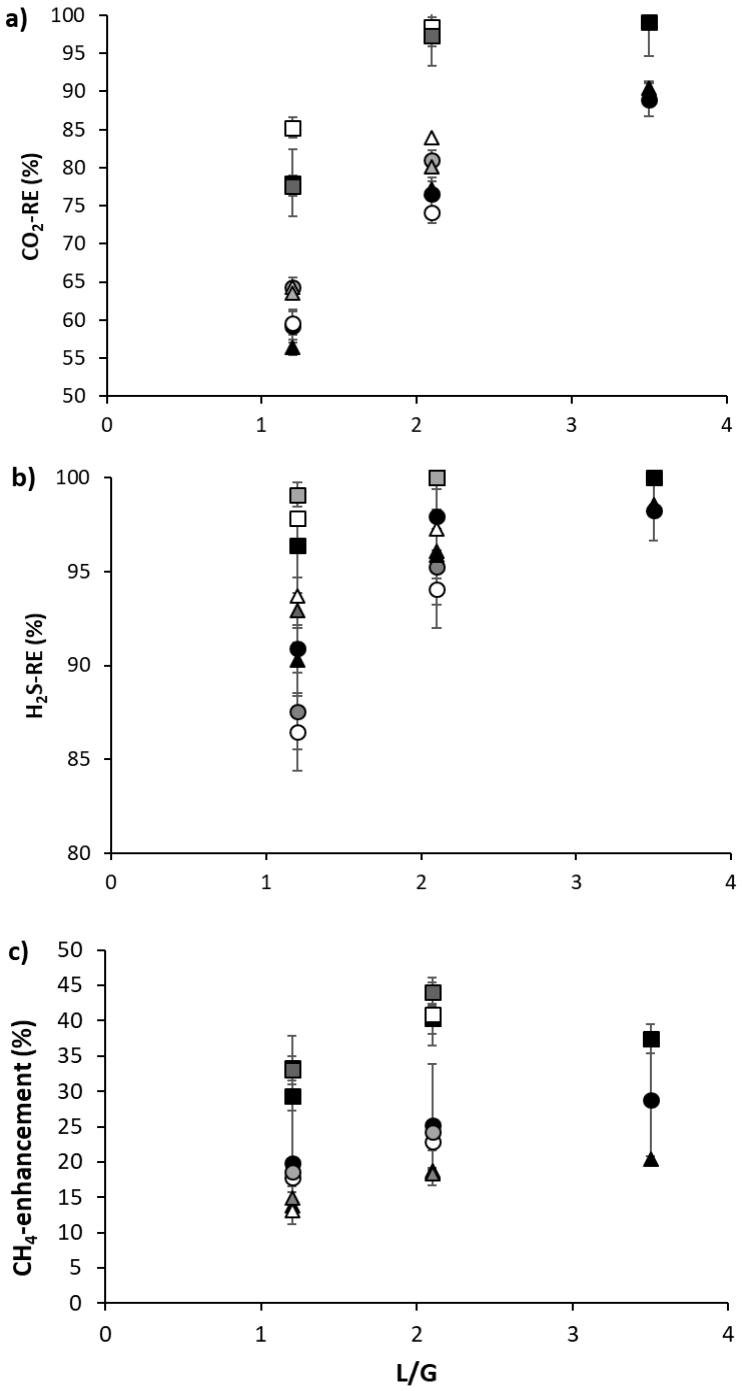

Figure 2. Influence of the L/G ratio on the (a) removal efficiency of CO_2, (b) removal efficiency of H_2S and (c) CH_4 enhancement factor at a biogas flowrate of 274 (black), 370 (white) and 459 (grey) L h^{-1} during stage I (○), stage II (Δ) and stage III (□).

3.2.2. H_2S removal

H_2S-REs of 90.9±0.7, 97.9±0.1 and 98.2±0.2% were achieved during photosynthetic biogas upgrading at a L/G ratio of 1.2, 2.1 and 3.5, respectively, when operating at a biogas flowrate of 274 L h^{-1} during stage I (Figure 2b). Similarly, H_2S-REs increased

from 86.4±1.3 to 94.0±2.8% and from 87.6±2.9 to 95.2±1.2% when the L/G increased from 1.2 to 2.1 at biogas flowrates of 370 and 459 L h^{-1}, respectively, under process operation with domestic wastewater at 3.5 days of HRT. The highest H_2S removals were achieved at the highest L/G ratio as a result of the higher volumetric mass transfer coefficients and higher concentrations gradients (the latter supported by the higher pH in the absorption column mediated by the increased fresh recycling liquid flowrate). In addition, the significantly higher H_2S-REs compared to the elimination of CO_2 were attributed to the higher aqueous solubility of H_2S (dimensionless Henry's Law constant = C_L/C_G three times higher than that of CO_2) (Sander, 1999).

During stage II, H_2S-REs of 90.3±4.9, 95.9±5.4 and 98.5±0.4% were recorded at a L/G ratio of 1.2, 2.1 and 3.5, respectively, at a biogas flowrate of 274 L h^{-1} (Figure 2b). No significant influence of the HRT ($p > 0.05$) on H_2S-RE was observed when feeding the HRAP with domestic wastewater. On the other hand, H_2S-REs increased from 93.7±1.4 to 97.3±0.1% and from 92.9±1.0 to 96.1±0.8% when the L/G increased from 1.2 to 2.1 at a biogas flowrate of 370 and 459 L h^{-1}, respectively, under process operation with domestic wastewater at a HRT of 8 days.

Finally, H_2S-REs of 96.4±5.1, 97.8±0.3 and 99.1±1.3% were recorded at a L/G ratio of 1.2 and biogas flowrates of 274, 370 and 459 L h^{-1}, respectively, during stage III, while a complete removal was obtained when the L/G ratio was increased to 2.1 and 3.5 (Figure 2b). The increase in H_2S-REs observed during this stage, when centrate was used as a water and nutrient source, in comparison with those of stages I and II, was attributed to the higher pH and buffer capacity of the recirculating cultivation broth, which increased the gas-liquid mass transfer of H_2S due to its acidic nature. These results agreed with the observations of Rodero et al. (2018b), who recorded an increase in H_2S removal from 80.3 to 94.7% when the IC concentration of the cultivation broth increased from 100 to 500 mg L^{-1} at 12ºC and L/G ratio of 0.5 in a 180 L HRAP operated indoors.

3.2.3. Enhancement in the CH_4 content of the upgraded biogas

The CH_4 enhancement factor, defined as the ratio between the increase in CH_4 content (%CH_4 in biomethane - %CH_4 in raw biogas) and the CH_4 content (%) in raw biogas, was used to comparatively assess the influence of the L/G, biogas flow rate, type of wastewater and HRT. CH_4 enhancement factors of 19.9±8.4, 25.3±8.8 and 28.8±8.7%, which corresponded to CH_4 concentrations of 79.3±2.8, 83.7±1.8 and 86.8±1.8% in the

upgraded biogas, were recorded at L/G ratios of 1.2, 2.1 and 3.5, respectively, at a biogas flowrate of 274 L h^{-1} during stage I. Similarly, CH_4 concentration in the upgraded biogas increased from 81.2±0.1 to 84.7±0.6% (CH_4 enhancement factors of 17.8±1.6 and 22.8±0.9%) and from 81.6±0.6 to 85.6±0.2% (CH_4 enhancement factors of 18.6±0.1 and 24.3±0.6%) when L/G increased from 1.2 to 2.1 at biogas flowrates of 370 and 459 L h^{-1}, respectively (Figure 2c). The increase in L/G ratio played a key role on the CH_4 enhancement factor mediated by CO_2 and H_2S removals, while a negligible influence ($p>0.05$) of the biogas flowrate was recorded on CH_4 concentration in the upgraded biogas. However, the increase in L/G ratio also induced a higher desorption of the N_2 and O_2 dissolved in the cultivation broth to the biogas in the absorption column, thus decreasing the CH_4 concentration in the upgraded biogas (Posadas et al., 2017a). Indeed, the O_2 + N_2 concentration in the upgraded biogas increased up to 7.4±0.4% at a L/G ratio of 3.5 under process operation with domestic wastewater at a HRT = 3.5 days. The higher stripping of N_2 and O_2 at higher L/G ratios was due to the higher turbulence in the absorption column, which increased the overall liquid-gas mass transfer coefficients, and to the increase in the mass flow rate of these gases potentially stripped out to the biomethane (Serejo et al., 2015). In this context, O_2 and N_2 stripping could be limited by operating under low L/G ratios and conditions that selectively enhance CO_2 and H_2S gas-liquid mass transfer.

During stage II, CH_4 enhancement factors of 13.8±0, 13.2±0.6 and 15.0±1.3%, which corresponded to final CH_4 concentrations of 85.4±0.3, 85.1±0.7 and 87.0±0.9 were recorded at a L/G ratio of 1.2 and biogas flowrates of 274, 370 and 459 L h^{-1}, respectively (Figure 2c). An increase in CH_4 concentration up to ~89% was recorded at a L/G ratio of 2.1 regardless of the biogas flowrate and only a slight increase in CH_4 concentration up to 90.4±0.6% was obtained when the L/G ratio was increased to 3.5 (Table 2). Despite higher CH_4 concentrations in the upgraded biogas were recorded when the HRT of the domestic wastewater in the HRAP was increased from 3.5 to 8 days, lower CH_4 enhancement factors were achieved as a result of the higher CH_4 concentrations in the raw biogas in this stage (75.3±0.3 % in stage II *vs* 68.4±1.7 % in stage I).

During stage III, CH_4 enhancement factors of 29.4±5.0, 40.3±1.3 and 37.4±0%, which corresponded to CH_4 concentrations of 83.3±2.0, 90.3±2.2 and 88.2±2.2 in the upgraded biogas, were recorded at L/G ratios of 1.2, 2.1 and 3.5, respectively, at a biogas flowrate

of 274 L h^{-1} (Table 2). The increase in L/G ratio from 2.1 to 3.5 under process operation with centrate also resulted in lower final CH_4 concentrations due to the higher N_2 and O_2 desorption from the recycling liquid to the biomethane. Interestingly, higher $N_2 + O_2$ concentrations in the upgraded biogas (up to 11.4±2.0%) were recorded as a result of the increase in the overall mass transfer coefficients mediated by the higher ionic strength of the recycling liquid in stage III, which prevented the coalescence of the fine bubbles produced by the biogas diffuser (Sovechles and Waters, 2015). In our particular study, the maximum CH_4 content in the upgraded biogas (90.3%) remained below the minimum limit required for biogas injection in natural gas grid in the Spanish standard (95%) or the limit imposed by some car manufactures for use as a vehicle fuel. Nevertheless, an increase of the alkalinity in the cultivation broth would improve CO_2 and H_2S absorption, which would ultimately allow operating at lower L/G ratios (with the subsequent decrease in the O_2 content and increase in CH_4 content).

Table 2. Average composition of the upgraded biogas in the different operational stages

Stage	G (L h^{-1})	L/G	Upgraded biogas			
			CH_4 (%)	CO_2 (%)	H_2S (ppm_v)	N_2+O_2 (%)
I	274	1.2	79.3±2.8	17.3±2.2	167±119	3.3±1.5
	274	2.1	83.7±1.8	11.8±1.4	65±49	4.5±0.4
	274	3.5	86.8±1.4	5.8±1.0	40±42	7.4±0.4
	370	1.2	81.2±0.1	17.1±0.1	442±25	1.7±0.2
	370	2.1	84.7±0.6	11.6±1.1	205±92	3.7±0.5
	459	1.2	81.6±0.6	16.6±1.1	440±63	1.7±0.6
	459	2.1	85.6±0.6	10.0±0.9	190±42	4.5±0.7
II	274	1.2	85.4±0.3	15.8±0.8	18±12	-
	274	2.1	89.2±0.2	9.0±0.4	8±3	1.9±0.3
	274	3.5	90.4±0.6	4.3±0.2	3±0	5.3±0.8
	370	1.2	85.1±0.7	13.6±0.6	10±1	1.3±0.2
	370	2.1	89.1±0.4	7.0±0.1	5±0	3.9±0.3
	459	1.2	87.0±0.9	12.8±0.1	11±1	0.2±0.8
	459	2.1	89.5±0.0	7.3±0.2	6±0	3.2±0.2
III	274	1.2	83.3±2.0	10.1±4.4	65±92	6.6±2.5
	274	2.1	90.3±2.2	1.2±0.6	0±0	8.5±1.6
	274	3.5	88.2±2.2	0.5±0.2	0±0	11.4±2.0
	370	1.2	87.2±2.2	7.2±1.0	43±11	5.7±1.2
	370	2.1	90.6±0.7	0.9±0.8	0±0	8.6±0.1
	459	1.2	82.5±0.3	11.1±1.1	15±21	6.5±0.8
	459	2.1	89.3±0.7	1.8±0.3	0±0	8.9±0.5

3.3. Wastewater treatment performance

The COD-REs recorded in the HRAP accounted for 86.9±1.8, 90.7±4.1 and 73.6±0 %, which resulted in effluent COD concentrations of 85.8±10.3, 49.6±16.2 and 123.8±0 mg O_2 L^{-1} during stages I, II and III, respectively (Figure 3). The higher effluent COD concentrations in stage III compared to the previous stages were likely mediated by the higher HRT (process operation without effluent), which supported a higher biomass decay. However, effluent COD concentrations always complied with the Directive 98/15/CEE (125 mg O_2 L^{-1} maximum COD concentration for wastewater discharge into the environment) regardless of the type of wastewater or HRT ("Directive 98_15_CEE," 1998).

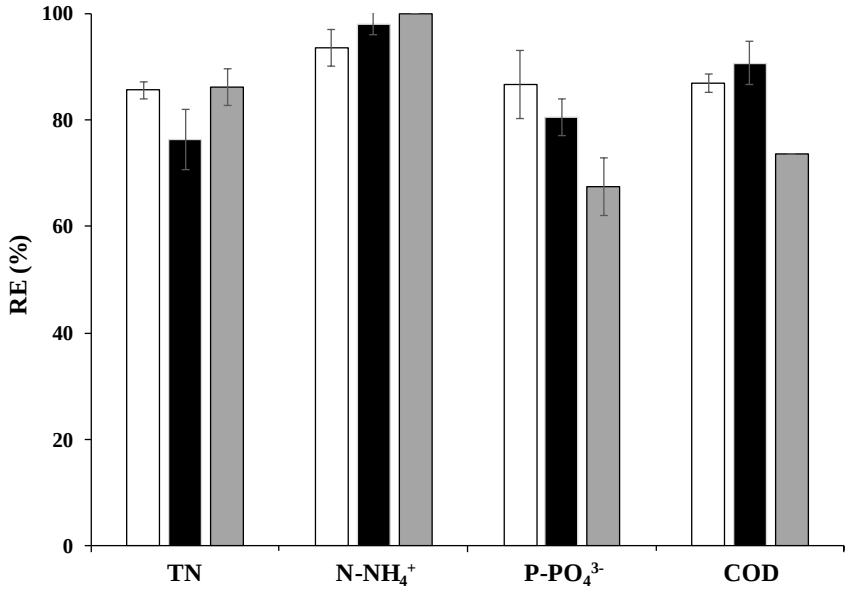

Figure 3. Steady state removal efficiencies of total nitrogen (TN), ammonium ($N\text{-}NH_4^+$), phosphate ($P\text{-}PO_4^{3-}$) and chemical oxygen demand (COD) during stage I (white), II (black) and III (grey).

High $N\text{-}NH_4^+$ REs were achieved during the three stages (93.6±3.5, 98.1±2.1 and 100±0% in stages I, II and III, respectively). However, the removals of TN under steady state were lower and averaged 85.6±1.6, 76.4±5.7 and 86.2±3.4% during stages I, II and III respectively (Figure 3). This mismatch between TN and $N\text{-}NH_4^+$ eliminations was caused by the active nitrification of a fraction of the inlet nitrogen to NO_2^- and NO_3^-. In this context, $N\text{-}NO_3^-$ was the dominant form of oxidized nitrogen since $N\text{-}NO_3^-$ effluent concentrations averaged 2.0±1.2, 9.6±0.5 and 38.1±7.4 mg L^{-1}, while $N\text{-}NO_2^-$ effluent concentrations averaged 0.8±0.5, 0.4±0.2 and 13.3±11.7 mg L^{-1} in stages I, II and III, respectively. The maximum fraction of the inlet nitrogen converted into $N\text{-}NO_2^-$+$N\text{-}NO_3^-$ was recorded during stage II (18.5%). These results agreed with Arcila and

Buitrón (2016), who recorded an incomplete nitrification or no nitrification when the HRT decreased from 10 to 6 days as a result of a nitrifying biomass wash-out. On the other hand, the lower share of nitrification during stage III compared to stage II was attributed to a high NH_4^+ volatilization mediated by the high pH (~9) under operation with centrate.

Finally, $P\text{-}PO_4^{3-}$-REs of 86.7±6.3, 80.6±3.5 and 67.6±5.4%, which entailed $P\text{-}PO_4^{3-}$ effluent concentrations of 1.0±0.5, 1.3±0.3 and 19.9±5.4 mg L^{-1} during stages I, II and III, respectively, were recorded (Figure 3). In this regard, these $P\text{-}PO_4^{3-}$-REs agreed with values previously reported in literature and highlighted the high bioremediation efficiency of HRAPs devoted to biogas upgrading (García et al., 2017; Toledo-Cervantes et al., 2016).

3.4. Concentration and elemental composition of the algal-bacterial biomass

TSS concentrations in the HRAP cultivation broth of 0.33±0.10, 0.37±0.08 and 0.56±0.05 g L^{-1} were recorded during stages I, II and III, respectively, with a similar VSS/TSS ratio of ~ 0.74. These TSS values were similar to those reported by Posadas et al. (2015b) (321-494 mg L^{-1}) in three outdoors HRAP treating domestic wastewater at 2.7-6 days of HRT under different pHs. The higher TSS concentration in the HRAP during stage III was attributed to the higher nutrient concentrations of the centrate compared to domestic wastewater.

The C and N content of the harvested biomass (on a dry weight basis) remained constant at 32.1±1.7 and 5.6±0.6%, respectively, regardless the operational stage. Despite this C content was lower compared to the typical range reported in literature for different microalgae strains (40-60 wt.%)(Teles et al., 2013), this value was in agreement with Muñoz et al. (2015b) who recorded a C content of 32.2% and 30.4% in the biomass of the strains *Botryococcus Braunii* and *Nannochloropsis gaditana*, respectively. Similarly, Harman-ware et al. (2013) reported a C content of 32.1% in *Scenedesmus sp.* biomass. The N content and the C/N ratio (5.7) in the harvested biomass remained within the range of previously reported data (Ward et al., 2014). The main differences were recorded in S content, which varied from 0.68±0.08% during stages I and II to 0.30±0.05% during stage III. These results agreed with those reported by Posadas et al. (2017a), who observed a decrease in S content in the biomass from 0.4% to 0.2% concomitantly with the increase in the IC concentration of the cultivation broth. The decrease in the S content of the algal-bacterial biomass recorded could be

attributed to the lower SO_4^{2-} loading rate during stage III (mediated by process operation at a higher hydraulic retention time). However, this phenomenon requires further investigation.

3.5. Biogas upgrading technology costs

Despite the fact the investment cost of photosynthetic biogas upgrading is ~1.5-2.2 times higher than that of conventional-physical chemical technologies, and a larger footprint is required (a total HRAP surface of ~13.4 ha is needed to treat 300 $Nm^3 h^{-1}$ of biogas considering a water depth of 0.2 m) (Toledo-Cervantes et al., 2017b), the environmental sustainability (CO_2 trapped in form of algal bacterial biomass and wastewater treatment), the simultaneous H_2S removal and the lower energy requirements and operating costs, make this technology an attractive alternative for biogas upgrading (Table 3). Moreover, algal-bacterial biomass valorization as a bio-fertilizer can outbalance the high investment costs of this innovative process.

Table 3. Biogas upgrading technology costs (Angelidaki et al. 2018, Marín et al. 2018; Muñoz et al. 2015, Toledo-Cervantes et al. 2017b)

	Water scrubbing	**Chemical scrubbing**	**Organic scrubbing**	**PSA**	**Membrane separation**	**Cryogenic separation**	**HRAP-AC**
Investment costs (€ $(Nm^3 h^{-1})^{-1}$)	3500	3200	4000	2700	2800	-	6000
Operating costs (€ Nm^{-3})	0.13	-	-	0.18	0.2	-	0.03
Energy requirements (kW-h Nm^{-3})	0.25-0.3	0.67-0.7	0.4-0.51	0.24–0.6	0.2-0.38	0.42-1	0.08-0.14
CH_4 content (%)	>96	96-99	96–98.5	96-98	96-98	>97	90
H_2S pretreatment	Recommended	Yes	Recommended	Yes	Recommended	Yes	No

4. Conclusions

This work constitutes, to the best of our knowledge, the first demo-scale validation of the simultaneous photosynthetic biogas upgrading and wastewater treatment under outdoor conditions. The type of wastewater played a key role on biogas upgrading (with higher CO_2 and H_2S removals using centrate due to its higher pH and alkalinity), while the influence of the HRT and biogas flowrate on biogas upgrading performance was negligible. Despite higher L/G ratios supported higher CO_2 and H_2S removals, the associated N_2 and O_2 stripping resulted in a lower biomethane quality. Finally, an efficient wastewater treatment was achieved regardless of the operational conditions.

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Chapter 6

Development of a control strategy to cope with biogas flowrate variations during photosynthetic biogas upgrading

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Development of a control strategy to cope with biogas flowrate variations during photosynthetic biogas upgrading

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ABSTRACT

The design and evaluation of a control system for a photosynthetic biogas upgrading unit was successfully carried out in this study. This control system ensured a specific biomethane quality under any disturbance in the biogas flowrate. The recycling liquid flowrate, and indirectly the liquid to biogas (L/G) ratio, was selected as the manipulated variable in order to maintain the CO_2 and O_2 content of biomethane, and therefore comply with the requirements for its use as natural gas substitute ($\leq$2.5% and $\leq$1.0%, respectively). The control system was able to maintain the biomethane CO_2 content below the set point value under a stepwise increase in the biogas flowrate from 60 to 150 ml min^{-1}, together with negligible H_2S concentrations and an O_2 stripping from the recycling liquid to the biomethane lower than 1%, thus obtaining a consistent biomethane quality over time. On the contrary, the biomethane CO_2 content increased up to 13.2% under this stepwise increase in the biogas flowrate without control system. Successful results were also obtained when the control system was challenged with stepwise surges in the biogas flowrate between 60 and 120 ml min^{-1} under different temperatures (15 and 35°C) and inorganic carbon concentrations (1500, 500 and 100 mg L^{-1}) when the recycling liquid entering the absorption column presented a pH=10. However, the high liquid flowrates required at a cultivation broth pH of 8.5 as a result of the low CO_2 mass transfer led to an excessive O_2 desorption to the biomethane, resulting in biomethane O_2 contents >1%.

Keywords: Algal-bacterial processes; biogas upgrading; biomethane; photobioreactor; process control.

1. Introduction

Biogas is a byproduct obtained from the anaerobic digestion of organic waste and wastewater. It is typically composed of CH_4 (40-75%), CO_2 (30-50%), H_2S (0.005-2%) and other pollutants at trace level concentrations, such as oxygen, nitrogen, ammonia, siloxanes and volatile organic compounds [1]. The high CH_4 content has encouraged the use of biogas as a bioenergy vector for the production of heat and power, and even as a substitute of natural gas. However, the presence of other components apart from CH_4 hinders its direct injection into the natural gas grids or its use as a vehicle fuel. For instance, CO_2 results in higher greenhouse gas emission during biogas combustion, increases biogas transportation costs and reduces its specific calorific value. Similarly, H_2S reduction is highly recommended due to its corrosive, malodorous and pernicious nature [2]. In this context, biogas upgrading prior use as a vehicle fuel or its injection into natural gas grids is a compulsory step which must ensure concentrations of $CH_4 \geq$ 90%, $CO_2 \leq$ 2-4%, $O_2 \leq$ 1% and trace levels of H_2S according to most international regulations [3,4].

Physical/chemical technologies for CO_2 removal often need a preliminary H_2S abatement stage and exhibit high energy and chemical requirements that jeopardize the economic viability of biomethane as a renewable substitute of natural gas. On the other hand, biological technologies such as biofiltration or *in situ* microaerobic digestion for H_2S removal coupled to hydrogenotrophic biogas upgrading for CO_2 removal always involve a two-stage process [5]. In this regard, photosynthetic biogas upgrading through algal-bacterial processes represents a cost-effective and environmentally sustainable alternative for the simultaneous CO_2 and H_2S removal [6]. During photosynthetic biogas upgrading, microalgae use solar light energy to capture the CO_2 present in biogas, while H_2S is oxidized to S^0/SO_4^{2-} by sulfur-oxidizing bacteria using the oxygen photosynthetically produced [7]. In addition, the nutrients required to support microalgal and bacterial growth in this technology can be obtained from wastewaters from different sources, which contributes to enhance its environmental sustainability [8]. Photosynthetic biogas upgrading is typically implemented in two interconnected units consisting of a bubble absorption column (AC) that removes the unwanted pollutants from the biogas and a high rate algal pond (HRAP) where the biological processes above described occur.

Several works have evaluated the performance of photosynthetic biogas upgrading coupled to wastewater treatment under indoor conditions at a constant biogas flowrate [9–14]. However, the performance of anaerobic digestion is affected by multiple variables such as temperature, mixing regime, or feedstock composition and load, whose fluctuations could lead to changes in the daily biogas production and composition. These changes impact on the subsequent upgrading process and can compromise the quality of the biomethane produced [15–18]. Moreover, a recent study in an outdoors HRAP interconnected to an AC showed that the photosynthetic biogas upgrading performance is influenced by the environmental conditions prevailing throughout the year. Therefore, variations in the temperature, pH or alkalinity of the cultivation broth (i.e. associated to rain or evaporation) ultimately impact on the CO_2 and H_2S mass transfer in the AC and consequently on the biomethane quality [19]. Thus, the development of a control system for the photosynthetic biogas upgrading process is necessary in order to make the process more robust towards environmental or operational fluctuations, and to ensure a biomethane complying with most regulations for its use as a natural gas substitute.

In this context, the liquid to gas ratio (L/G) has been identified as an important operating parameter in gas-liquid mass transfer units [20,21]. An increase in the gas flow rate reduces the mass transfer between the two phases, which is attributed to both the lower gas residence time and bubble coalescence. Conversely, an increase in the liquid flow rate entails a higher gas absorption in the liquid phase due to the higher the contact area, but an enhanced stripping of compounds from the liquid to the gas phase [22]. For instance, Serejo et al. [23] observed an increase in CO_2 removal efficiency at increasing L/G ratios up to 15; while a complete H_2S removal was achieved regardless of the tested L/G ratio due to the higher H_2S aqueous solubility. Nevertheless, an increase in the L/G ratio in the biogas absorption column also resulted in a higher O_2 concentration in the upgraded biogas, due to an enhanced desorption of the dissolved oxygen from the microalgae cultivation broth [24]. In this regard, the L/G ratio in the AC is a key operational parameter that must be optimized during photosynthetic biogas upgrading in order to guarantee consistent CO_2 and O_2 concentration in the biomethane.

This study aimed at designing and evaluating the performance of a control system for biogas upgrading in a HRAP interconnected to an AC to cope with fluctuations in biogas production over time. The process response against variations in biogas flowrate

under different environmental conditions (alkalinity, pH and temperature) was assessed with and without control system.

2. Materials and methods

2.1. Experimental set-up

The experimental set-up was composed of an indoor 180 L HRAP interconnected to a 2.5 L AC via external liquid recirculation of the supernatant from a 10 L settler (Fig.1). The HRAP was continuously fed at 3 L d^{-1} with a mineral synthetic medium (pH 10) that simulated the composition of a high strength digestate from the anaerobic digestion process. The mineral medium had the following composition (g L^{-1}): 7.60 $NaHCO_3$, 3.70 Na_2CO_3, 0.58 K_2HPO_4, 1.91 NH_4Cl, 0.10 $MgSO_4 \cdot 7H_2O$, 0.02 $CaCl_2 \cdot 2H_2O$, 0.005 $FeSO_4 \cdot 7H_2O$ and 5 mL of a trace element solution prepared according to the *Spirulina* mineral salt medium recommended by the SAG Culture Collection [25]. The HRAP was continuously illuminated at ~1350 µmol m^{-2} s^{-1} and agitated at an internal recirculation velocity of ~20 cm s^{-1}. Synthetic biogas composed of 70% CH_4, 29.5% CO_2 and 0.5% H_2S (which is a typical composition of biogas obtained from the anaerobic digestion of sewage sludge or agro-industrial bio-waste [5,26]) was sparged co-currently with the recycling liquid into the AC via a metallic gas diffuser of 2 µm pore size located at the bottom of the AC.

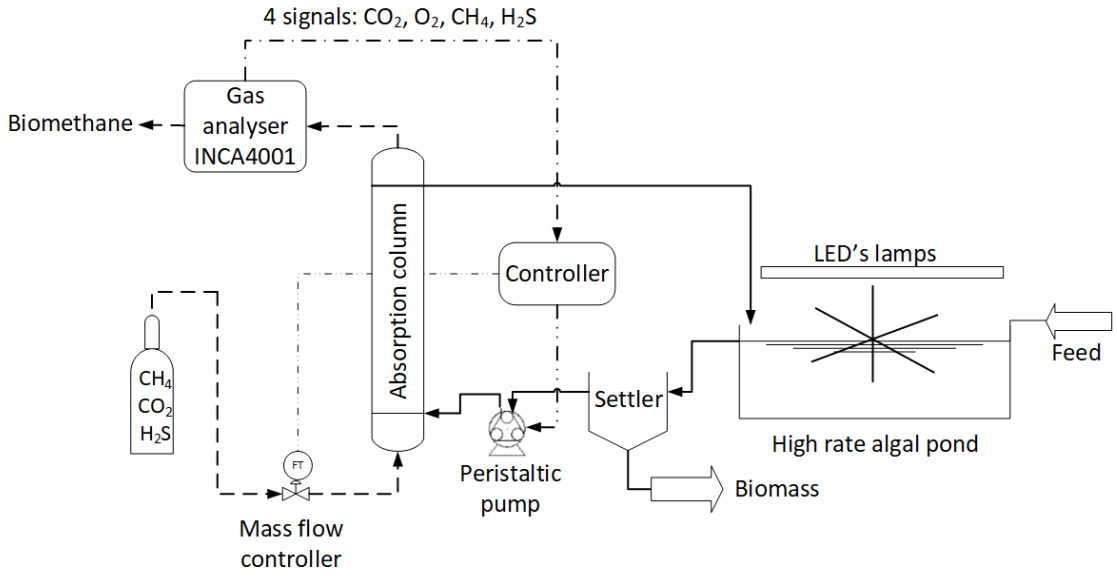

Fig. 1. Experimental set-up and control layout for photosynthetic biogas upgrading

The biogas flow rate variations were conducted using a mass flow controller (Aalborg, USA) connected to a synthetic biogas cylinder (Fig.1). The recycling liquid flow rate in the AC was pumped using a variable flow peristaltic pump DINKO D-25Vplus (Spain).

The system was operated under steady state at an initial L/G ratio of 0.5 based on previous studies and at a constant liquid flow rate of 30 ml min^{-1} [27]. The upgraded biogas was accumulated in a Tedlar bag prior measuring its composition (CH_4, CO_2, H_2S and O_2 content) in an online gas analyzer INCA 4001 (UNION Instruments GmbH, Germany). The control unit was composed of a field-programmable gate array (FPGA) «myRio 1900» via an interface developed in LabVIEW 2014 (National Instruments). The pH of the cultivation medium was determined using a pH meter Eutech Cyberscan pH 510 (Eutech instruments, The Netherlands).

2.2. Control system design

A rule-based control method aiming at maintaining a biomethane quality over time under biogas flow rate fluctuations was developed. The control rules were designed based on previous observations. This type of control was selected because of the constraints imposed by the analyzer (with a sampling time of 1-2 hours), which prevented the use of standard control methods such as PID. Moreover, a rule-based control allowed taking advantage of the practical experience accumulated by the research team in the operation of this type of plants. In addition, the system was non-linear and time-varying, which would require the use of some type of gain-scheduling with the associated problems of tuning under different operating conditions. The CO_2 content in the upgraded biogas was chosen as one of the controlled variables since H_2S removal efficiency (RE) is typically higher than CO_2-RE due to the ~3 times higher H_2S Henry's Law constant (C_L/C_G), while CH_4 losses in the absorption column are negligible due to its low aqueous solubility [28]. Additionally, the O_2 content in the upgraded biogas resulting from the desorption of dissolved O_2 in the AC was the other controlled variable taken into account since a high concentration of O_2 in biomethane can result in explosive mixtures [29]. O_2 and CO_2 concentrations in the biomethane were fixed at a set point of 1% and 2.5%, respectively, in order to comply with most international regulations. The manipulated variable was the recycling liquid flow rate, which determines the L/G ratio in the AC (Fig.2).

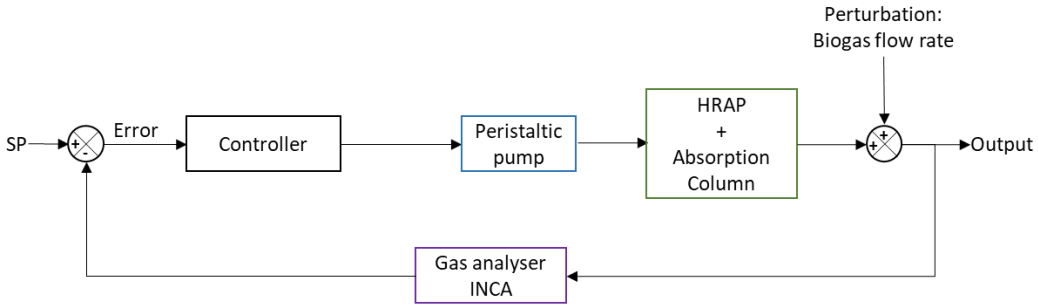

Fig. 2. Block diagram of the control system

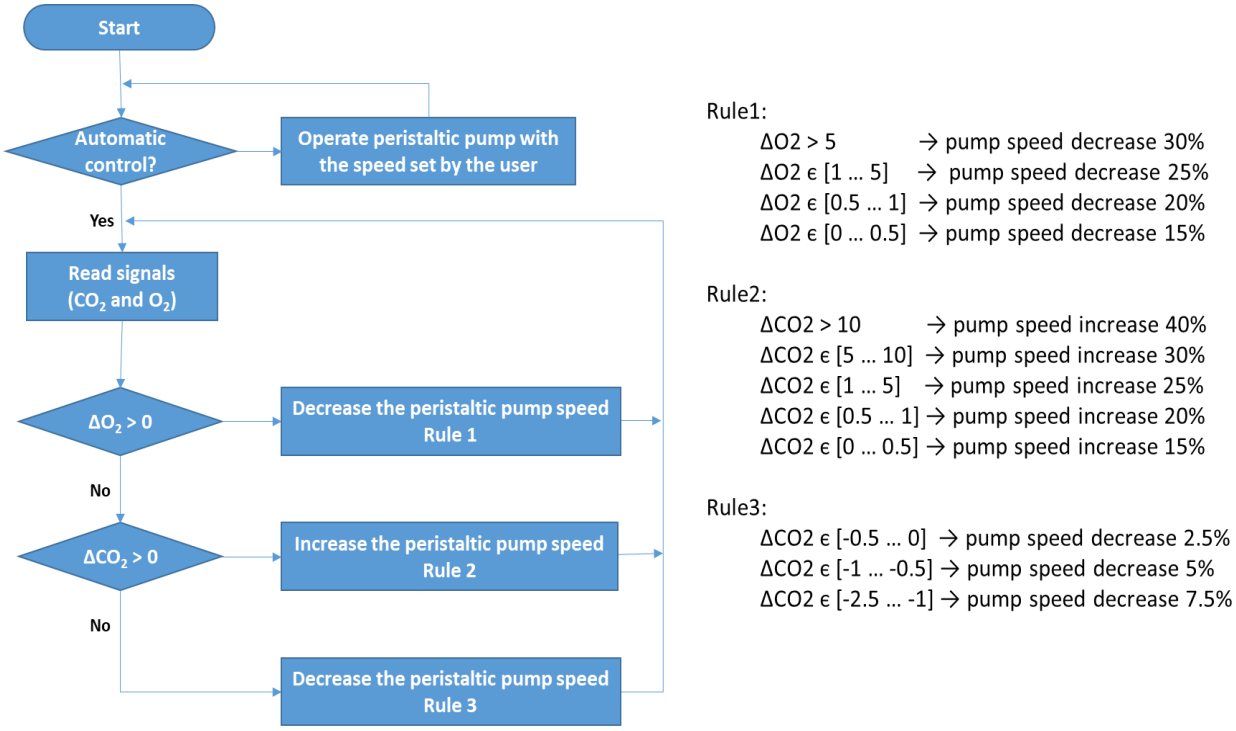

Fig. 3. Flow diagram of the ruled-based control system and rule values

Fig. 3 shows the rules of the control system where $\Delta CO_2 = [CO_2]_{measured} - [CO_2]_{sp}$, $\Delta O_2 = [O_2]_{measured} - [O_2]_{sp}$; the value "measured " being the one obtained from the gas analyser and the set point (sp) the value fixed based on the target values of most international regulations. When the O_2 content in the biomethane was higher than 1% (set point value) (rule 1), the flow rate of the liquid pump was decreased even if the CO_2 content in the upgraded biogas was higher than the set point CO_2 concentration due to safety reasons. When O_2 content in the biomethane was < 1% and CO_2 content > 2.5%, the control system increased the flow rate of the recycling liquid pump in order to enhance CO_2 absorption (rule 2). In the case of rule 3, when CO_2 and O_2 concentration in the upgraded biogas complied with the set-point, the flow rate of the recycling liquid pump was decreased in order to save energy. In this context, the amount of change in

the recycling liquid flow rate was variable depending on the values of the variables involved (O_2 and CO_2 concentration in the upgraded biogas) as shown in Fig.3.

2.3. Step response of the control system interconnected to a HRAP

The proposed control system was evaluated under different perturbations in the biogas flow rate in order to test its effectiveness and robustness. First, a 4 h step increase from G = 60 to 150 ml min^{-1} and back to 60 ml min^{-1} was carried out to test the response of the system under biogas flow rate surges. Secondly, a similar step with a higher duration was implemented in order to ensure that the control system was able to maintain the steady state. Finally, the biogas flow rate was stepwise increased by 10 ml min^{-1} every 2 hours from 60 to 120 ml min^{-1} in the first 12 h and decreased to 60 ml min^{-1} within the next 12 h. This simulated real fluctuations in a biogas production process. The composition of the upgraded biogas accumulated in the Tedlar bag was measured every two hours prior actuation of the control system, except in the case of the biogas flowrate of 150 ml min^{-1} where measurements were conducted every hour. These sampling times were selected based on the sampling volume requirements by the biogas analyzer and the low value of the biogas flows used in this laboratory scale set-up. All the experiments consisted of two similar consecutive biogas flowrate cycles under controlled and uncontrolled (without any change in the recycling liquid flowrate) conditions, in order to evaluate the effectiveness and significance of the control system. The values of the changes implemented in the liquid flowrate depending on the CO_2 and O_2 concentrations are summarized in Table S1 (Supplementary data).

2.4. Validation of the control system at varying biogas flowrates under different environmental conditions

Process response to the stepwise variations in biogas flowrate (stepwise variations from 60 to 120 ml min^{-1} for 12 h and from 120 to 60 ml min^{-1} for the next 12 h) was validated under controlled and uncontrolled conditions at different temperatures (15 and 35°C), pH (10 and 8.5) and inorganic carbon (IC) concentrations (1500, 500 and 100 mg L^{-1}) in the recycling liquid. The experiments were carried out in duplicate. For this purpose, a similar mineral medium, with different concentrations of $NaHCO_3$ and Na_2CO_3 to achieve the desired IC concentration and pH, was used as recycling liquid in the absorption column. The temperature of the recycling liquid before entering the absorption column was adjusted using an external heat exchanger (Fisherbrand™ Polystat™ Immersion Circulator, Germany) and the temperature in the absorption

column was maintained using an external coil connected to a heat exchanger (Huber CC1-E Immersion-Thermostat Control, Germany). A different set of variations in the recycling liquid flowrate (power pump changes) were used during these experiments due to the necessity of changing the pipe of the peristaltic liquid pump for the highest flowrate requested during some of these assays (Table S2 Supplementary data).

3. Results and discussion

3.1. Step response of the control system

Figure 4 shows the response of CO_2 and H_2S concentration in the upgraded biogas and pH in the recirculating broth at the outlet of the absorption column under controlled and uncontrolled conditions during a 4 hours biogas flowrate step increase from 60 to 150 ml min^{-1}, and back to 60 ml min^{-1}, along with the liquid flow rate during the control period. The performance of the experimental system was significantly affected when biogas flow rate was increased from 60 to 150 ml min^{-1} for 4-h. Hence, CO_2 concentration in the upgraded biogas increased from 1.5 to 10.7%, which corresponded to a CO_2-RE decrease from 95 to 64%, concomitantly with the 4-h step increase in the biogas flowrate when the control system was not running (Fig. 4a). Similarly, an increase in the H_2S content from zero to 400 ppm_v in the upgraded biogas (which corresponded to a H_2S-RE decrease from 100 to 92%) was observed as a result of the surge in biogas flow rate (Fig. 4b). This deterioration of the system performance was recorded in spite of the high alkalinity of the cultivation broth (~2500-3000 mg IC L^{-1}), which was associated to an IC accumulation mediated by water evaporation and the high strength medium used as nutrient source in the HRAP. The increase in the biogas flowrate (×2.5) at a constant liquid flowrate resulted in a decrease in the L/G ratio from 0.5 to 0.2, which likely mediated CO_2 and H_2S saturation of the recycling cultivation broth with the subsequent decrease in the pH along the AC. In this context, the pH decreased from a value of 10 at the bottom of the AC to 9.6 and 8.4 at the top of the AC at biogas flowrates of 60 and 150 ml min^{-1}, respectively (Fig. 4c). This drop in the pH along the AC resulted in a lower CO_2 and H_2S gas-liquid mass transfer due to the decrease in the concentration gradient of these acidic gases in the liquid phase. The O_2 content in the upgraded biogas remained almost constant at ~0.2%, as a result of the constant liquid flowrate and the low L/G ratios. Likewise, Toledo-Cervantes et al. [14] reported O_2 concentrations in the biomethane below 0.1% in a similar indoor system at L/G ratios ranging from 0.3 and 0.5.

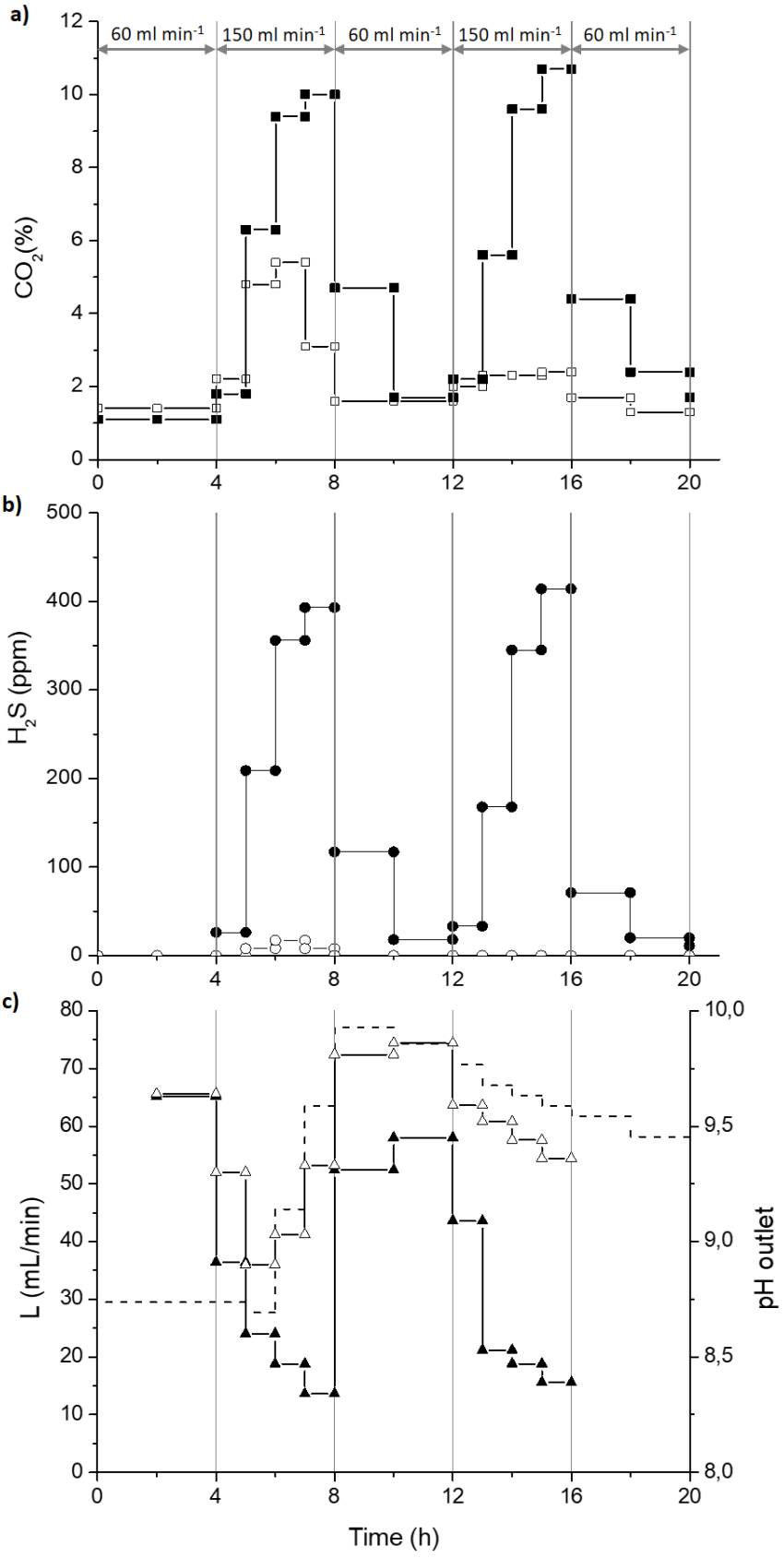

Fig. 4. Time course of a) CO_2 content, b) H_2S content of the upgraded biogas and c) liquid flow rate (dashed line) and pH at the outlet of the absorption column under controlled (open) and uncontrolled (solid) conditions during the 4-h biogas flowrate step increase experiment.

When the control system was initiated, CO_2 content of the upgraded biogas increased up to 5.4% (~2 times less than that without control) during the first surge in biogas flowrate to 150 ml min^{-1}, and remained under the set point value during the duration of the second flowrate step (Fig. 4a). The lower CO_2 content recorded in the upgraded biogas during the latter step could be explained by the higher liquid flowrate (L) imposed by the control system prior to the second surge in biogas flowrate. The H_2S content of the upgraded biogas during this experiment was negligible regardless of the biogas flowrate, due to its high solubility in water and the effectiveness of the proposed control system (Fig. 4b). The higher CO_2 and H_2S-REs achieved when the control system was active could be attributed to the lower acidification of the cultivation broth between the bottom and the top of the AC as a result of the lower amount of CO_2 and H_2S transferred per volume of recycling liquid when the liquid flow rate in the AC was actively controlled. Moreover, the O_2 content in the upgraded biogas remained under the set point value along the entire period. Overall, the maximum L/G ratio recorded was 1.3 at a liquid flowrate of 77 ml min^{-1} (Fig. 4c), which ensured a good biomethane quality (CH_4 content >95%) during most of the experiment.

When the step increase in biogas flowrate was maintained for 12 h in order to confirm the ability of the system to maintain a steady state over time, the CO_2 content in the upgraded biogas increased up to 13.2% when the control system was not active (Fig. 5a). The lower CO_2 content in the upgraded biogas observed during the 4-h step test confirmed that the system was not able to reach steady state at a biogas flowrate of 150 ml min^{-1}. In this context, only ~4-5 h after the step increase in the biogas flowrate, the CO_2 content in the upgraded biogas remained almost constant. On the contrary, the maximum H_2S content obtained in this experiment was 230 ppm_v lower than during the 4-h step test (Fig. 5b). The increase in H_2S removal during this experiment could be attributed to a higher dissolved oxygen (DO) concentration in the cultivation broth and/or bacteria activity during these days, which ultimately enhanced H_2S oxidation. Unfortunately, data of DO or pH in the cultivation broth of the HRAP was not continuously recorded and this hypothesis could not be fully confirmed.

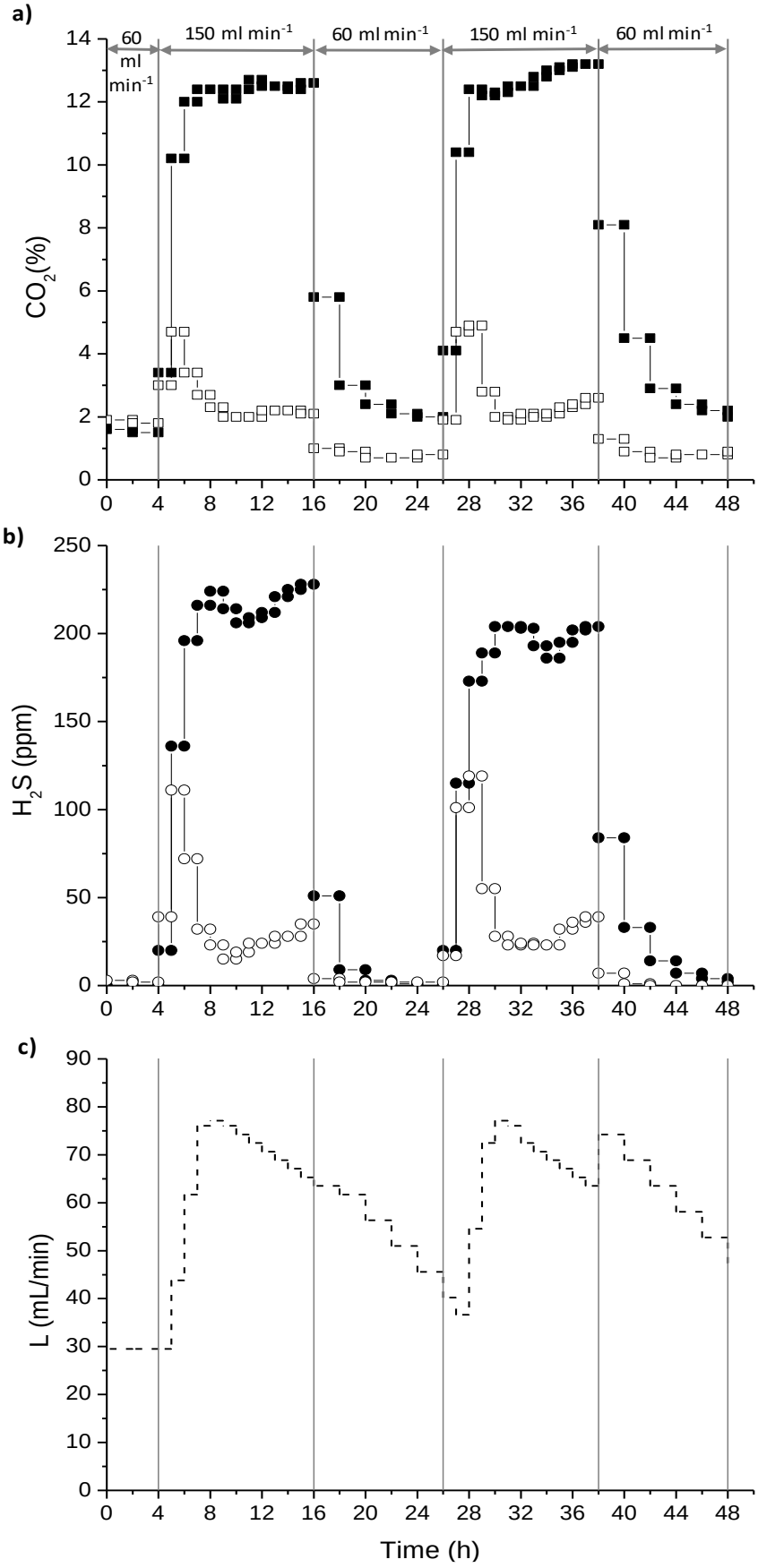

Fig. 5. Time course of a) CO_2, b) H_2S content of the upgraded biogas under controlled (open) and uncontrolled (solid) conditions and c) liquid flowrate during the 12-h biogas flowrate increase step experiment.

The control system showed a similar performance regardless of the duration of the biogas flowrate step increase: a maximum CO_2 content of 4.9% in the upgraded biogas (~2.7 times lower than that without control) was achieved in both step increases from 60 to 150 ml min^{-1} (Fig. 5a), which correlated with the similar L/G ratios recorded when increasing the biogas flowrate. In addition, the H_2S content in the upgraded biogas reached 120 ppm_v with the increase in the biogas flowrate, obtaining a nearly complete removal afterwards (H_2S-RE >99%) (Fig. 5b). Likewise, the O_2 concentration remained under the set point value during both experiments with and without control system. Moreover, identical maximum liquid flowrate values (77 ml min^{-1}) and consequently L/G ratios (1.3) were obtained in both step increase experiments (Fig. 5c). The lower CH_4 concentration recorded during the step increase was 93.4% and approximately three hours after the step (the control system had acted 3 times), a suitable biomethane quality (CH_4 content >95%) was achieved. The results revealed that the implementation of a control system in a large-scale biogas upgrading unit would entail a faster and even more accurate process response as a result of the shorter time between measurements.

The biogas flowrate was also stepwise increased by 10 ml min^{-1} every 2 h from 60 to 120 ml min^{-1}. Without the control system, the CO_2 concentration in the upgraded biogas increased up to 7.8%, already exceeding the CO_2 set point (2.5%) at a biogas flowrate of 90 ml min^{-1} (corresponding to L/G ratios < 0.33) (Fig. 6a). These results were in accordance with Toledo-Cervantes et al. [14], who recorded CO_2-REs of 70.3 and 97.3% at L/G ratios of 0.3 and 0.5, respectively, operating under co-current mode under a similar high pH and alkalinity of the cultivation broth than those tested in this study. When the biogas flowrate stepwise decreased from 150 to 60 ml min^{-1}, the CO_2-RE slowly increased due to the previous acidification of the cultivation broth, and the system was not able to recover the initial biomethane quality (CO_2 content ≤ 2.5%) even at the lowest biogas flowrate of 60 ml min^{-1}. In addition, the H_2S content in the upgraded biogas increased up to 280 ppm_v (Fig. 6b), while the O_2 remained lower than the set point value (1%) as in the previous experiments.

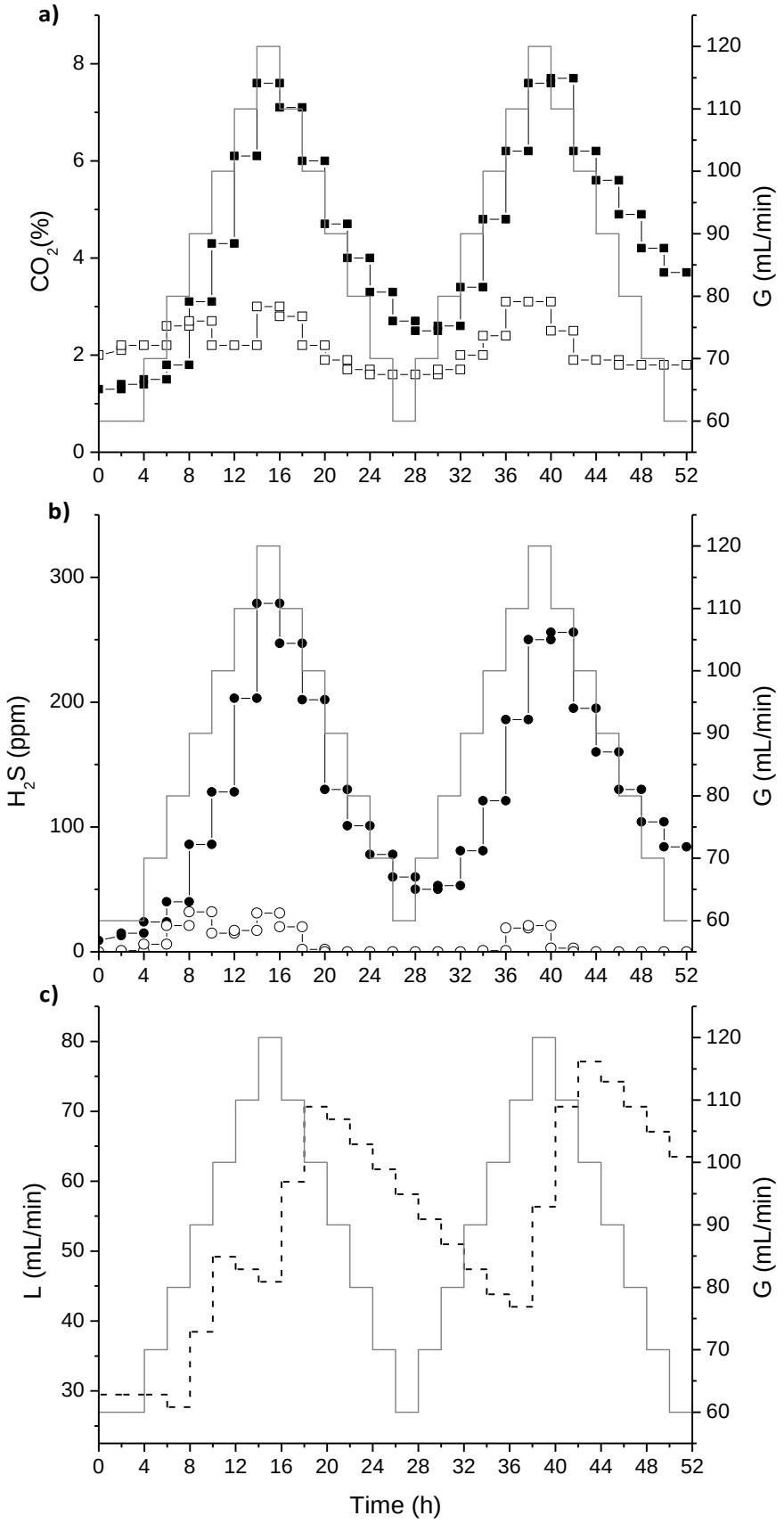

Fig. 6. Time course of a) CO_2, b) H_2S content of the upgraded biogas under controlled (open) and uncontrolled (solid) conditions and c) liquid flow rate (dashed line) during the stepwise biogas flowrate increase (continuous line) by 10 ml min^{-1} from 60 to 120 ml min^{-1}.

The maximum CO_2 concentration in the upgraded biogas when the control system was active was 3.1% (~2.5 times lower than that without control). A value lower than the set point was obtained after two control actions (Fig. 6a). The lowest CO_2-RE recorded was 89.5%, compared to the lowest value of 73.6% observed without control. In accordance with the results obtained without control, the CO_2 content in the upgraded biogas exceeded the set point value when the L/G ratio was lower than 0.38. Furthermore, the H_2S content in the biomethane was negligible regardless the biogas flowrate, which confirmed the robustness of the control system for H_2S removal using the CO_2 content in upgraded biogas as controlled variable. O_2 content in the biomethane remained below 1% with a maximum liquid flowrate and L/G ratio of 77 ml min^{-1} and 1.1, respectively (Fig. 6c). Finally, CH_4 concentration in the upgraded biogas was >94 % during the complete experimentation period, thus demonstrating the effectiveness of the control system even if the biogas flowrate variations occurred as sequential steps of lower magnitude.

Overall, the control strategy implemented in the experimental set-up consisting of a HRAP interconnected with an AC was able to maintain the operational variables below the set-points under multiple biogas flowrate surges, thus providing the required biomethane quality during most of the experimental period. However, the response of the system when operating under different environmental conditions (mediated by seasonal changes) could be different. Therefore, a further validation of the control system was carried out by assessing the upgrading performance at different alkalinities, pHs and temperature values.

3.2. Validation of the control system under different environmental conditions

3.2.1. Alkalinity

The alkalinity of the cultivation broth has been previously identified as a key parameter on CO_2 and H_2S removal in photosynthetic biogas upgrading. A high alkalinity medium results in a high buffer capacity and; consequently, in improved CO_2 and H_2S mass transfer rates as a result of the low decrease in the pH along the absorption column [13]. In this context, high strength digestates or agroindustrial wastewaters (i.e. piggery wastewaters) could be used to achieve an effective photosynthetic biogas upgrading since they usually contain high inorganic carbon concentrations (~1500 mg L^{-1}) [30,31]. For instance, Marin et al. [24] supplemented a carbonate solution to the AC in order to increase the alkalinity of the recycling liquid, and improved the CO_2 and H_2S-REs when

the photobioreactor was fed with an agricultural wastewater with a low IC concentration (36 mg L^{-1}). However, carbonate dilution might occur due to rainfall or no carbonate addition in outdoor systems. Then, the use of medium strength digestates (~500 mg IC L^{-1}) or domestic wastewaters (~100 mg IC L^{-1}), which are typically found in wastewater treatment plants, is the most common operating alternative. Under these scenarios, a decrease in the upgrading process efficiency could occur, the validation of the control system under different alkalinity conditions being necessary [19].

The stepwise increase in biogas flowrate from 60 to 120 ml min^{-1} without control system resulted in maximum CO_2 contents in the upgraded biogas of 13.4, 18.0 and 19.6%, while H_2S concentration reached 552, 1440 and 2033 ppm_v at a pH of 10 and IC concentrations of 1500, 500 and 100 mg L^{-1}, respectively (Fig. S1 – Supplementary Material). The highest CO_2 and H_2S removals were obtained at the highest alkalinity content (1500 mg IC L^{-1}), while the CO_2 content in the upgraded biogas at lower alkalinities was higher than the set point value even at the lowest biogas flowrate. The system performance was significantly improved when the control system was turned on. Immediately after the increase in biogas flowrate to 70 ml min^{-1}, the CO_2 content in the upgraded biogas exceeded the set point except for the experiment at 1500 mg IC L^{-1}. In the assays at IC concentrations of 500 and 100 mg L^{-1}, the control system increased the recycling liquid flowrate to 50 and 57 ml min^{-1}, respectively, based on the values of the previously stablished rules (Table S1). As a consequence, the highest CO_2 concentrations recorded in the upgraded biogas were 3.7, 4.2 and 5.1% at IC concentrations of 1500, 500 and 100 mg L^{-1}, respectively. These results demonstrated that when the control system was active, the influence of the alkalinity on the upgrading performance was significantly reduced (Fig. 7a). Similarly, the maximum H_2S content in the upgraded biogas was 12, 184 and 331 ppm_v at 1500, 500 and 100 mg IC L^{-1}, respectively (Fig. 7b). On the other hand, no significant O_2 concentration was measured in the upgraded biogas (<1%) even at 100 mg IC L^{-1}. Maximum liquid flowrates of 57, 99 and 99 ml min^{-1}, corresponding to maximum L/G ratios of 0.5, 1.1 and 1.1, were recorded at 1500, 500 and 100 mg IC L^{-1}, respectively. It is important to notice that, although similar maximum liquid flowrates were set at 500 and 100 mg IC L^{-1}, the highest flowrate was maintained during longer periods of time at the lowest alkalinity (Fig. 7c). These results agreed with Bahr et al. [6], who recorded an O_2 content in the biomethane below 1% at a L/G 1.2 regardless of the pH.

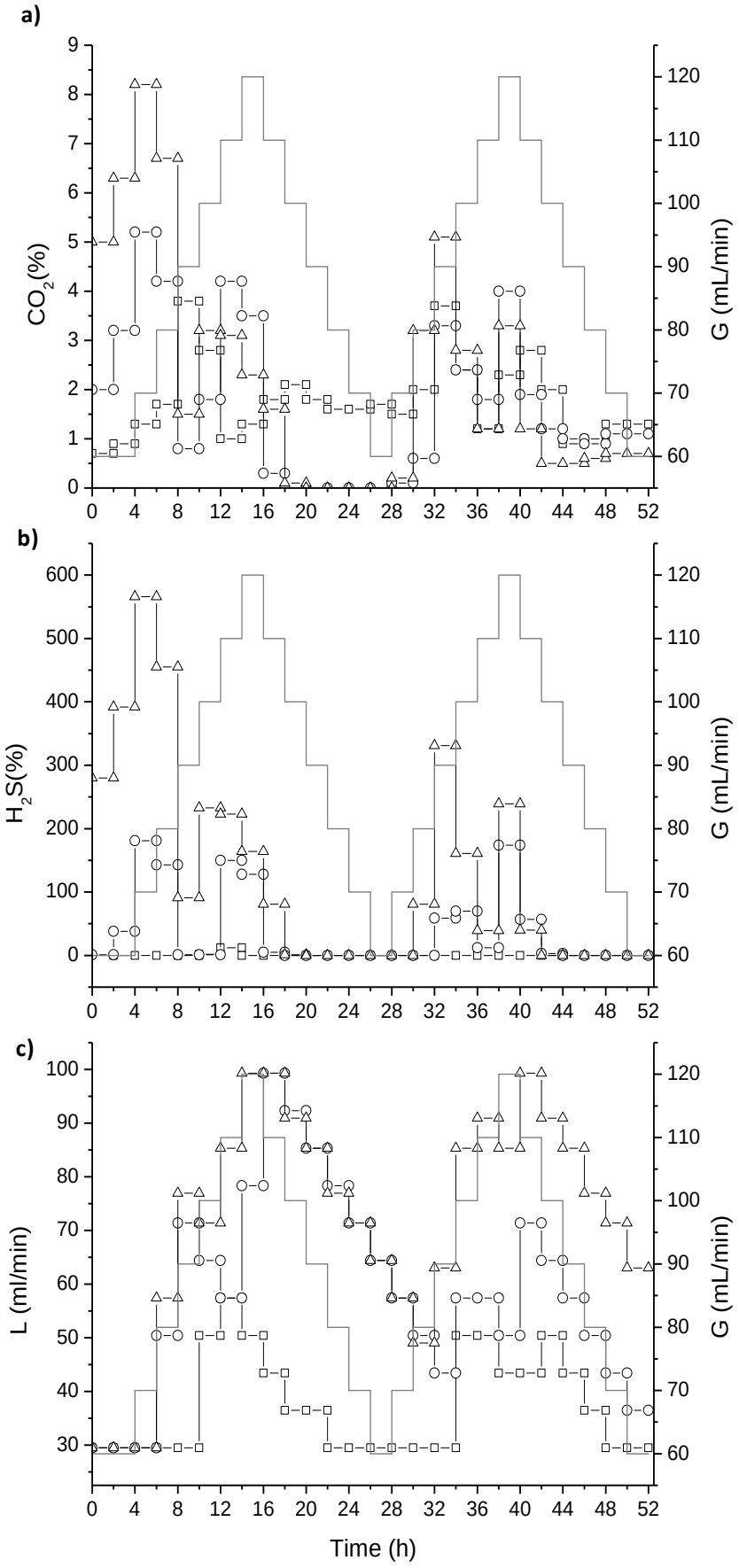

Fig. 7. Time course of a) CO_2, b) H_2S content in the upgraded biogas and c) liquid flow rate under controlled conditions at IC concentration of 1500 (square), 500 (circle) and 100 mg L^{-1} (triangle).

3.2.2. pH

pH also exerts a high influence on CO_2 and H_2S removal in the absorption process due to the significant improvement of the solubility of these gases at high pH values. Under optimal conditions of alkalinity in the cultivation broth, typically encountered in high strength digestates (pH>9, 1500 mg IC L^{-1}), a high pH value (up to 11) is expected in the cultivation broth of a photosynthetic biogas upgrading unit as a result of the pH increase mediated by CO_2 uptake during microalgal photosynthesis [30,32,33]. Nevertheless, a continuous, long-term exposition to high biogas flowrates could lead to the acidification of the cultivation broth even at this high alkalinity. In this sense, the performance of the control system was assessed under high alkalinity at two different pH values (10 and 8.5).

In spite of the high alkalinity of the recycling liquid, CO_2 content in the upgraded biogas under uncontrolled conditions increased up to 21.9% at pH 8.5, corresponding to CO_2-RE of 25.8%, while the maximum CO_2 concentration recorded at a pH 10 was 13.4% (Fig. S2). Indeed, the minimum CO_2 concentration recorded under these conditions and pH 8.5 was 16% (greater than the highest CO_2 value during the experiment at pH 10). In the case of H_2S, the highest concentration recorded was 941 ppm_v at pH 8.5 versus 12 ppm_v at pH 10 (Fig. S2). These results highlight the key role of the operational pH in the absorption process of these acidic gases, and were in agreement with Bahr et al. [6], who recorded CO_2 removals lower than 20% at pH 7 and almost a complete CO_2 removal at pH 10 regardless of the liquid flowrate.

As a result of the lower CO_2-REs at pH 8.5 and, consequently, the high difference between the CO_2 measured and CO_2 set point when the control system was turned on, the increase in the flowrate of the recycling liquid pump was higher compared to other assays, reaching 204 and 211 ml min^{-1} during the first and second biogas surges, respectively (Fig. 8d). Therefore, at L/G ratios > 1.5, the O_2 content in the upgraded biogas increased over the O_2 set point value (1%). Hence, the recycling liquid flowrate was reduced by the control system during the next step, regardless of the CO_2 concentration in the upgraded biogas due to the priority of the established rules. As a result, the CO_2 content during these assays did not comply with the established set point value since the O_2 content increased when increasing the liquid flowrate (Fig. 8c). These results were in accordance with Marin et al. [24], who recorded an increase in the content of N_2+O_2 in the upgraded biogas from ~5 to ~12% at increasing the L/G ratio

from 1 to 2. Nevertheless, the control system mediated a decrease in the CO_2 content to 4.4% (CO_2-RE of 85%), which was 3.5 times lower than the lowest value recorded without the control system (Fig. 8a). Moreover, the maximum H_2S concentration in the upgraded biogas under these conditions was 238 ppm_v, H_2S being completely removed during most of the time (Fig. 8b).

3.2.3. Temperature

Temperature is an important environmental variable, which has to be taken into account specially when operating outdoor systems. This variable has a significant influence on gas solubility (decreasing with the increase in the temperature), the ionic equilibria, and consequently, the pH [34]. Moreover, temperature affects microalgae and bacteria growth, the optimal temperature for microalgae activity being between 15 and 35ºC, depending on the strain [35]. Therefore, the control system was evaluated under two representative temperatures typically found during autumn-spring and summer in mild climates.

Under uncontrolled conditions, the CO_2 and H_2S concentrations in the upgraded biogas reached values of 11.4 and 11.7% and 393 and 305 ppm_v at 15 and 35ºC, respectively (Fig. S3). The similarity between the values recorded at both temperatures was attributed to the high alkalinity of the cultivation broth. These results were in agreement with Rodero et al. [13], who demonstrated the negligible influence of the temperature at high alkalinity of the cultivation broth, while at low alkalinity, lower temperatures enhanced CO_2-REs. Similarly, when the control system was turned on, the highest CO_2 content in the upgraded biogas was 4.7 and 4.4% at 15 and 35ºC, respectively, while almost a complete H_2S removal was obtained regardless of the liquid flowrate and temperature (Fig. 9a, b). Finally, similar liquid flowrates were needed during the experiments (highest liquid flowrate of 64 ml min^{-1} at 35ºC vs. 57 ml min^{-1} at 15ºC), resulting in low O_2 concentrations <1% consistent with the low L/G ratios (<0.6) (Fig. 9c).

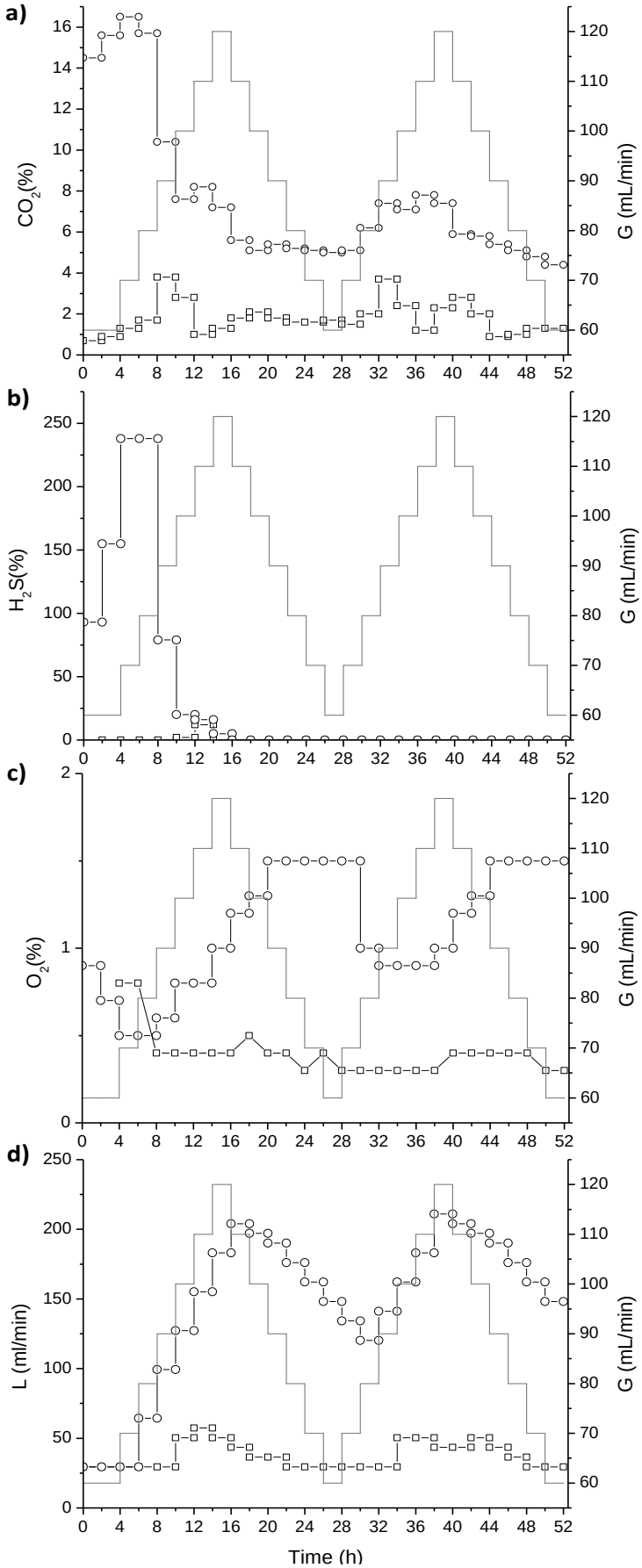

Fig.8. Time course of a) CO_2, b) H_2S c) O_2 content in the upgraded biogas and d) liquid flow rate under controlled conditions at pH 10 (square) and 8.5 (circle).

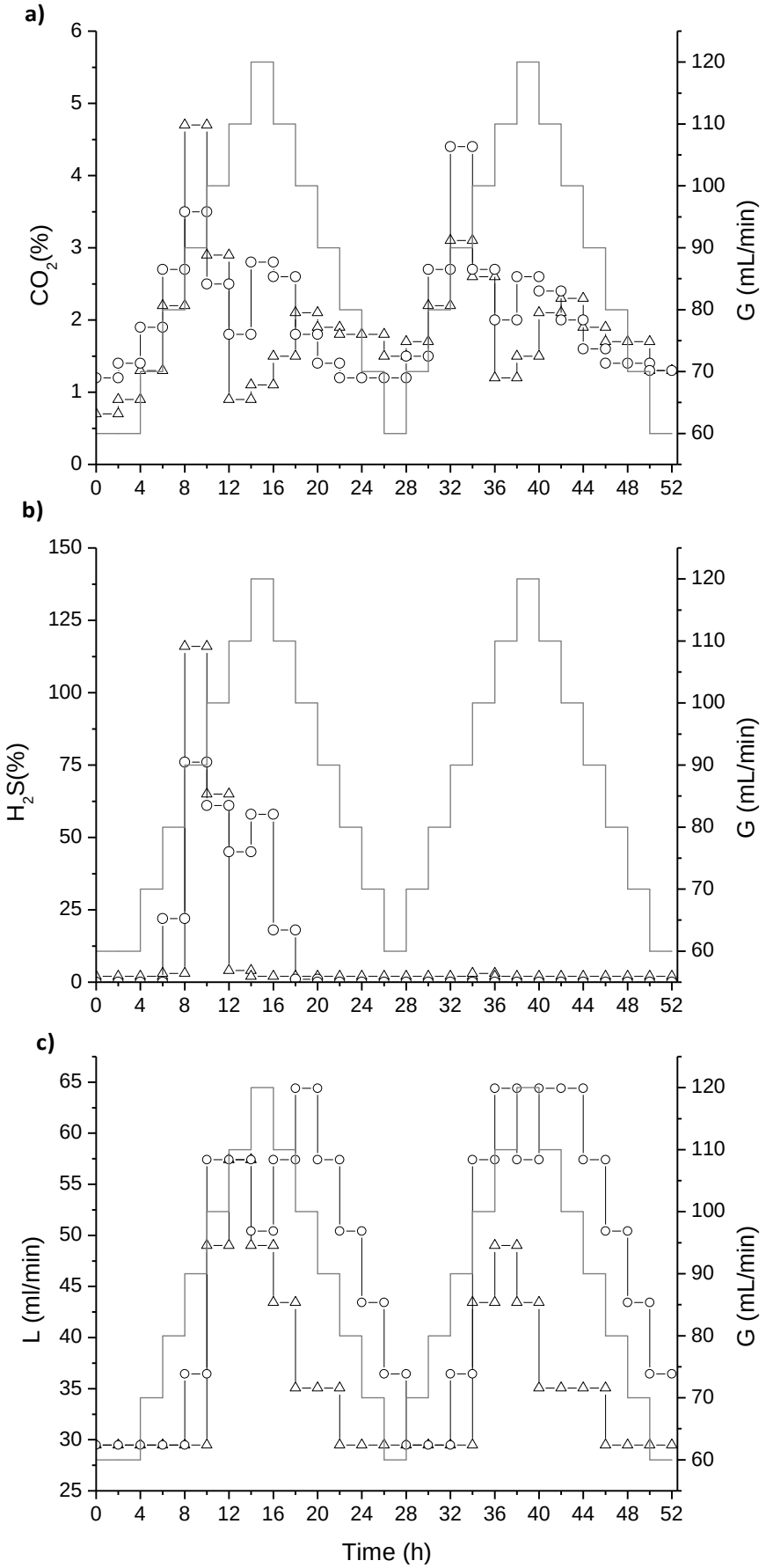

Fig. 9. Time course of a) CO_2, b) H_2S content in the upgraded biogas and c) liquid flow rate under controlled conditions at 35 (circle) and 15 ºC (triangle).

4. Conclusions

The recycling liquid flowrate was identified as a key operational variable in the control of the CO_2 and O_2 content in the upgraded biogas during photosynthetic biogas upgrading. The control system developed was capable of guaranteeing a CO_2 content lower than 2.5% during most of the experimental period regardless of the temperature and the alkalinity of the cultivation broth. Moreover, the O_2 remained lower than 1% and negligible concentrations of H_2S were recorded, obtaining a CH_4 concentration in the upgraded biogas >94%. On the contrary, the target biomethane quality was not achieved at a pH 8.5 due to the concomitant increase of both the O_2 and CO_2 concentrations in the upgraded biogas requiring opposite control strategies, confirming that pH was a critical operating parameter in these systems. In summary, the control system was effective under most tested laboratory conditions assuring an optimal liquid flowrate over time at low investment costs, although further optimization and validation under outdoor conditions and demo scale is still required.

Acknowledgements

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Supplementary Material

Development of a control strategy to cope with biogas flowrate variations during photosynthetic biogas upgrading

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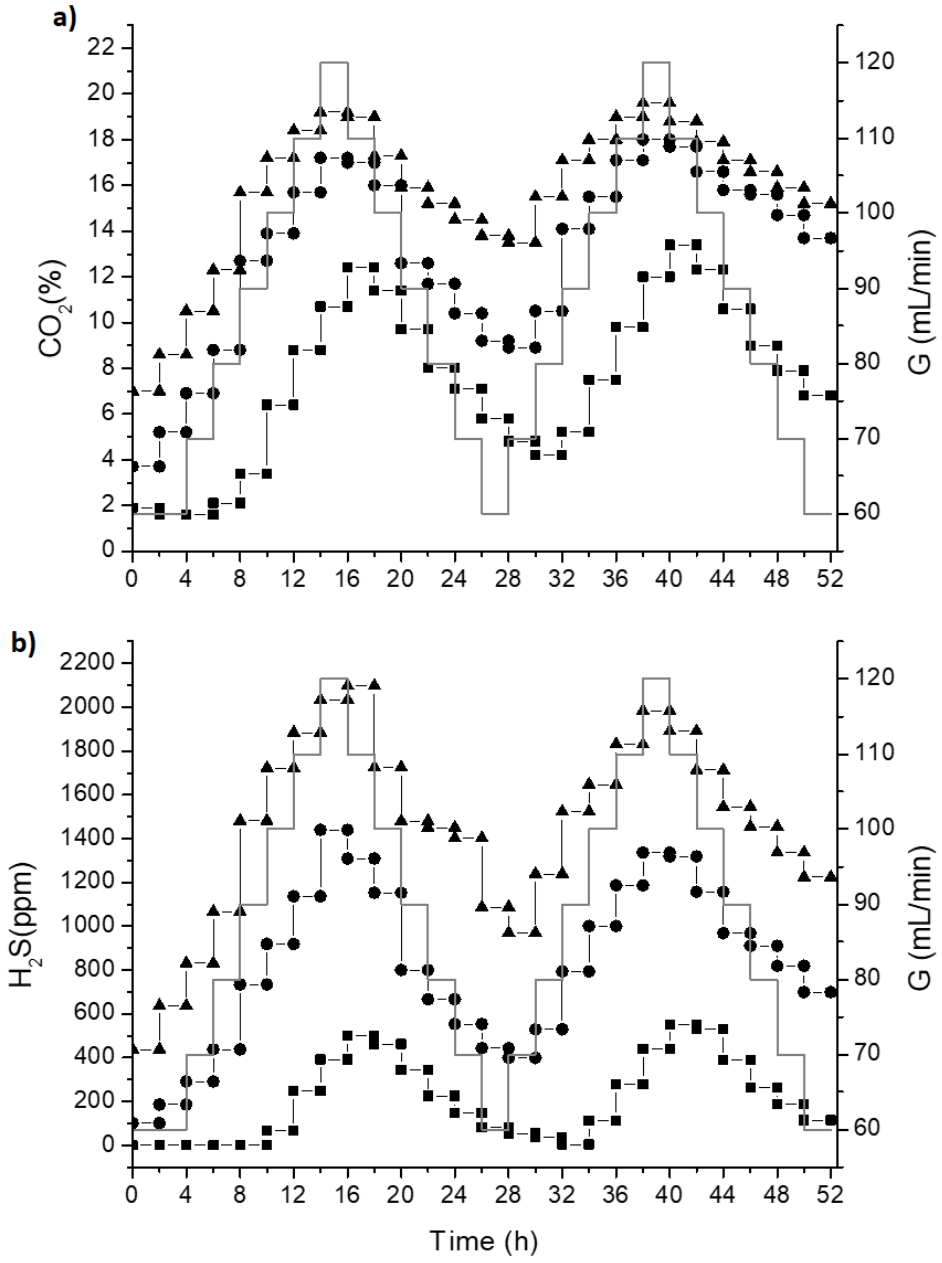


Figure S1. Step response of a) CO_2 and b) H_2S content in the upgraded biogas under uncontrolled conditions at IC concentration of 1500 (square), 500 (circle) and 100 mg L^{-1} (triangle). The continuous line represents the biogas flowrate (G).

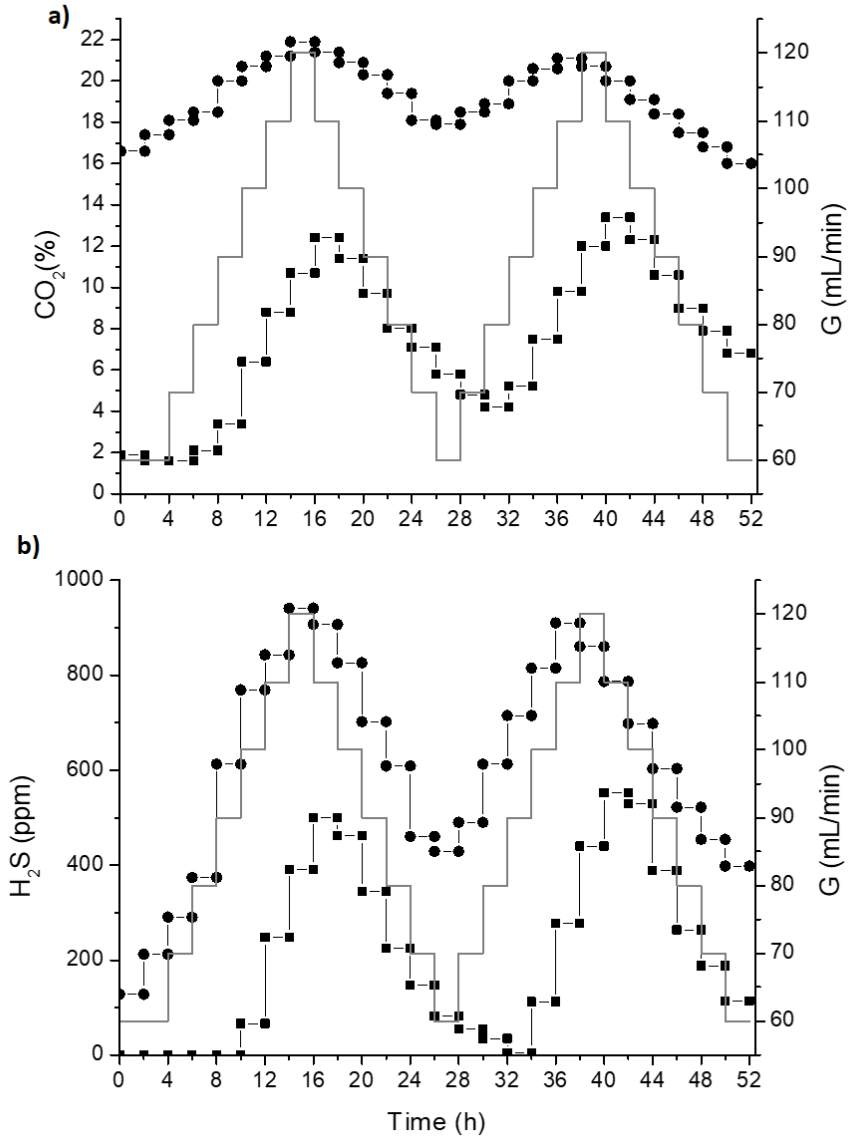


Figure S2. Step response of a) CO_2 and b) H_2S content in the upgraded biogas under uncontrolled conditions at pH 10 (square) and 8.5 (circle). The continuous line represents the biogas flowrate (G).

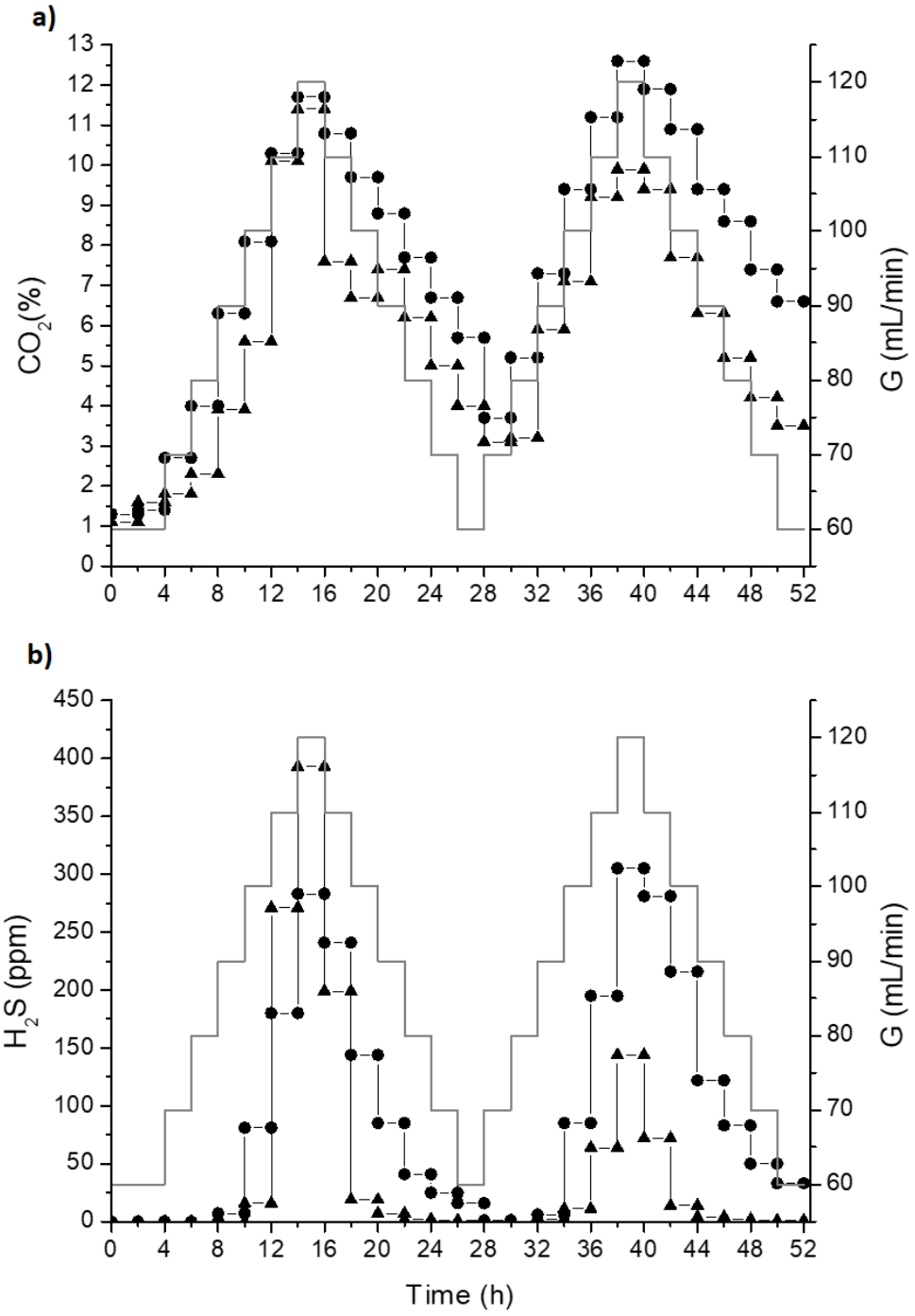


Figure S3. Step response of a) CO_2 and b) H_2S content in the upgraded biogas under uncontrolled conditions at 35 (circle) and 15 ºC (triangle). The continuous line represents the biogas flowrate (G).

Table S1. Variations in the recycling liquid flowrate under different concentrations of CO_2 and O_2 in the outlet biomethane during the step response of the control system.

Rule	ΔO_2	ΔCO_2	Power pump change (%)	Liquid flowrate change (mL min^{-1})
1	>5	-	-30	-21.6
	[1-5]		-25	-18.0
	[0.5-1]		-20	-14.4
	[0-0.5]		-15	-10.8
2	≤0	>10	40	28.8
		[5-10]	30	21.6
		[1-5]	25	18.0
		[0.5-1]	20	14.4
		[0-0.5]	15	10.8
3		[(-0.5)-0]	-2.5	-1.8
		[(-1)-(-0.5)]	-5	-3.6
		[(-2.5)-(-1)]	-7.5	-5.4

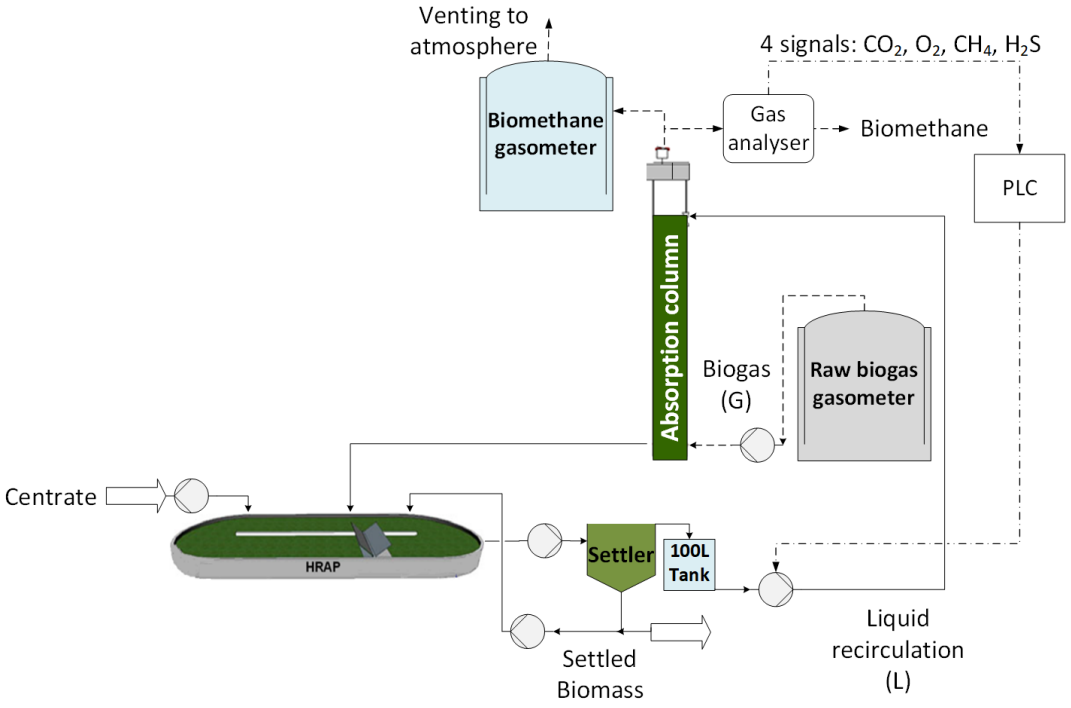
Table S2. Variations in the recycling liquid flowrate under different concentrations of CO_2 and O_2 in the outlet biomethane during the validation of the control system under different environmental conditions.

Rule	ΔO_2	ΔCO_2	Power pump change (%)	Liquid flowrate change (mL min^{-1})
1	>5	-	-10	-28
	[1-5]		-7.5	-21
	[0.5-1]		-5	-14
	[0-0.5]		-2.5	-7
2	≤0	>10	12.5	35
		[5-10]	10	28
		[1-5]	7.5	21
		[0.5-1]	5	14
		[0-0.5]	2.5	7
3		[(-0.5)-0]	0	0
		[(-1)-(-0.5)]	-2.5	-7
		[(-2.5)-(-1)]	-2.5	-7

Chapter 7

Performance evaluation of a control strategy for photosynthetic biogas upgrading in a semi-industrial scale photobioreactor

Rodero, M. del R., Carvajal, A., Arbib, Z., Lara, E., de Prada, C., Lebrero, R., Muñoz, R., 2020. Bioresour. Technol. 307, 123207. doi:10.1016/j.biortech.2020.123207



Performance evaluation of a control strategy for photosynthetic biogas upgrading in a semi-industrial scale photobioreactor

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ABSTRACT

The validation of a control strategy for biogas upgrading via light-driven CO_2 consumption by microalgae and H_2S oxidation by oxidizing bacteria using the oxygen photosynthetically generated was performed in a semi-industrial scale (9.6 m^3) photobioreactor. The control system was able to support CO_2 concentrations lower than 2% with O_2 contents ≤ 1% regardless of the pH in the cultivation broth (ranging from 9.05 to 9.50). Moreover, the control system was efficient to cope with variations in biogas flowrate from 143 to 420 L h^{-1}, resulting in a biomethane composition of CO_2 < 2.4%, CH_4 >95.5%, O_2 <1% and no H_2S. Despite the poor robustness of this technology against failures in biogas and liquid supply (CH_4 concentration of 67.5 and 70.9% after 2 h of biogas or liquid stoppage, respectively), the control system was capable of restoring biomethane quality in less than 2 h when biogas or liquid supply was resumed.

Keywords: algal-bacterial processes; biogas upgrading; biomethane; process control; semi-industrial scale.

1. Introduction

Biogas from the anaerobic digestion of the organic matter present in solid waste, wastewater or energy crops constitutes a valuable source of renewable energy. This green gas can be used for heat and/or power generation due to its high CH_4 content (50-75%) (Surendra et al., 2014). Nevertheless, the presence of contaminants such as CO_2 (30-50%) and H_2S (0.005-2%) hinders the widespread use of this sustainable energy vector (Ryckebosch et al., 2011). In this regard, the removal of CO_2 reduces biogas transportation and compression costs and increases its specific calorific value (Yan et al., 2016). On the other hand, H_2S removal is required since it is a hazardous and corrosive gas that promotes emissions of sulfur oxides (SO_x) during combustion (Brito et al., 2017). In this context, biogas upgrading is a mandatory step to enable its use as vehicle fuel or its injection into natural gas grids, which requires concentrations in biogas of $CH_4 \geq 90\%$, $CO_2 \leq 2\text{-}4\%$, $O_2 \leq 1\%$ and trace levels of H_2S according to most international regulations (Muñoz et al., 2015). The recast Renewable Energy Directive (RED II) sets an overall EU target to achieve at least a 32% consumption of energy from renewable sources by 2030, which includes an annual increase of 1.3% in the share of renewable energy in the heating sector and the use of a minimum of 14% renewable energy in the transport sector by 2030 (Directive (EU) 2018/2001, 2018). Therefore, biomethane has become increasingly attractive in Europe during the past years, where the number of biogas upgrading plants has increased from 187 to 540 in the 2011-2017 period, with a biomethane production up to 19352 GWh in 2017 (*EBA*, 2018). However, a cost-competitiveness and sustainable biogas upgrading technology is still necessary to boost the use of this promising energy source.

Nowadays, physicochemical methods such as water/organic/chemical scrubbing, pressure swing absorption and membrane separation for CO_2 removal are widely applied for biogas upgrading (*EBA*, 2018). However, these technologies often need a previous H_2S/siloxane/H_2O abatement step and exhibit a high energy and chemical demand that jeopardize the environmental and economic feasibility of biomethane (Awe et al., 2017). On the other hand, biological biogas upgrading require a two-step process (microaerobic digestion or biofiltration for H_2S removal followed by hydrogenotrophic CO_2 bioconversion into CH_4) and a surplus of electricity from renewable sources (to produce the H_2 required for microbial CO_2 reduction) (Angelidaki et al., 2018; Muñoz et al., 2015). In this context, photosynthetic biogas upgrading is an attractive alternative

for the concomitant and cost-competitive removal of CO_2 and H_2S from biogas (Nagarajan et al., 2019). This process is based on the fixation of CO_2 by microalgae in the presence of light and the oxidation of H_2S to S^0/SO_4^{2-} by sulfur-oxidizing bacteria using the oxygen produced by microalgal photosynthesis (Sun et al., 2016). Moreover, digestate from anaerobic digestion, a nutrient-rich effluent from the process, can be used as N and P source to support microalgal/bacterial growth, which improves the environmental and economic sustainability of this green technology (Ouyang et al., 2015).

The optimization of photosynthetic biogas upgrading coupled with nutrient recovery from digestates, which is commonly implemented in a bubble biogas scrubbing column (AC) interconnected via culture broth recirculation to a photobioreactor where the absorbed CO_2 and H_2S uptake occurs, has been carried out under indoors conditions at lab scale (Bahr et al., 2014; Franco-Morgado et al., 2017; Meier et al., 2018; Rodero et al., 2018; Serejo et al., 2015). Nevertheless, the performance of outdoors systems is governed by the daily and seasonal variations in environmental conditions, the pH in the cultivation broth being a critical parameter that impacts on both H_2S and CO_2 gas-liquid mass transfer in the AC (Bose et al., 2019; Posadas et al., 2017). In addition, the efficiency of the upgrading process could be affected by variations in the daily production and composition of biogas, process shutdowns or technical failures in equipment. In this regard, Rodero et al. (2019) designed a control system to cope with possible disturbances during photosynthetic biogas upgrading based on the optimization of the liquid to biogas ratio (L/G), which is a key factor determining the CO_2 and H_2S absorption in the AC (Meier et al., 2019). The control system was systematically evaluated in a 180 L high rate algal pond (HRAP) interconnected to an AC under indoors conditions with promising results under most conditions tested (biomethane composition of O_2<1% and CO_2<2.5% and CH_4>94%) (Rodero et al., 2019). However, the validation of any control strategy at a demo scale under outdoors conditions is a requirement prior full-scale implementation of this technology.

This study constitutes, to the best of our knowledge, the first evaluation under outdoors conditions and semi-industrial scale of the performance of a control system devoted to maintain or restore biomethane quality under environmental variations (different pH of the cultivation broth, daily biogas production fluctuations) or operational failures during photosynthetic biogas upgrading.

2. Materials and methods

2.1. Experimental set-up

The experimental set-up was composed of a 9.6 m^3 HRAP with an illuminated surface of 32 m^2 and a depth of 0.3 m, interconnected to a 7 m^3 conical settler prior to a 150 L biogas AC via an external recirculation of the cultivation broth. The system was operated outdoors during summer conditions (average ambient temperature and light radiance of 24.2±2.0 ºC and 25.5±1.3 MJ m^{-2} d^{-1}, respectively) at Chiclana de la Frontera WWTP (36.42°N, 6.15°W) (Spain). The HRAP consisted of two water channels divided by a central wall made of concrete blocks and two flow rectifiers in each loop to avoid dead zones, backflow and eddies (de Godos et al., 2016). The HRAP was continuously agitated at an internal liquid recirculation velocity of ≈30 cm s^{-1} by a 6-blade paddlewheel. The average composition of the real centrate, fed at a flow rate of 160 L d^{-1}, was (mg L^{-1}): alkalinity ($CaCO_3$) =2420±192, chemical oxygen demand (COD) = 793±214, total nitrogen (TN) = 724±118, ammonium ($N\text{-}NH_4^+$) = 579±27, phosphate ($P\text{-}PO_3^{4-}$) = 60±17 and volatile suspended solids (VSS) = 320±248. The inorganic carbon (IC) concentration of the HRAP cultivation broth was adjusted to 1907±109 mg L^{-1} by addition of $NaHCO_3$ and Na_2CO_3.

The algal-bacterial biomass was harvested from the bottom of the settler at a rate providing a fixed biomass productivity of 30 g m^{-2} d^{-1}. The algal-bacterial biomass was continuously produced (from CO_2, H_2S and nutrient fixation) and harvested, with a fraction being recirculated. This process, and the stability of the algal-bacterial biomass, was confirmed during a recent one-year round evaluation of the technology conducted by the authors (Marín et al., 2018).

Biogas, obtained from the anaerobic digestion of sewage sludge in a 20 m^3 digester located at Chiclana de la Frontera WWTP, was sparged into the AC using a polypropylene fine bubble diffuser (ECOTEC, Spain) under countercurrent flow configuration with the clarified cultivation broth (pumped from the top of the settler). Raw biogas composition was 70.5±1.7% CH_4, 31.5±1.1% CO_2 and 52±57 ppm H_2S. The low content of H_2S in the inlet biogas was mediated by the pretreatment performed to the sewage sludge prior anaerobic digestion. Biogas composition (CO_2, CH_4, O_2 and H_2S) was measured using an online gas analyzer INCA 4001 (UNION Instruments GmbH, Germany). The resolution of the sensors of the biogas analyzer was 0.1 vol.%

for CO_2, O_2 and CH_4 and 1 ppm_v in the case of H_2S. The range of measurement was 0-100 vol.% for CO_2 and CH_4, 0-25 vol.% for O_2 and 0-10000 ppm_v for H_2S, while the accuracy was ±1%, ±1%, ±3% and ±10% of the range for CO_2, CH_4, O_2 and H_2S, respectively. The control module was composed of a Programmable Logic Controller (PLC) "S7-315" via an interface developed using the software Human Machine Interface (HMI) Scada "WinCC Flexible 2008 SP4" (Siemens). The pH of the recycling liquid was measured using a Crison pH 4603 probe coupled to a Crison Multimeter 44 display (Barcelona, Spain). The concentration of dissolved IC in the cultivation broth was determined by means of a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module.

2.2. Control system strategy

A rule-based control system was implemented in order to maintain a biomethane quality over time according to the results reported by Rodero et al. (2019) during the evaluation of the control system under lab scale indoors conditions. The controlled variables were the O_2 and CO_2 concentration in the biomethane, while the manipulated variable was the recycling liquid flow rate, which consequently modified the L/G ratio in the AC. A set point value of 2% and 1% were set for CO_2 and O_2 concentrations, respectively, in order to comply with the target values for biomethane use as natural gas substitute in most international legislations (including the recent European Standard UNE-EN 16723). The O_2 content in biomethane was also selected as controlled variable since a high O_2 desorption in the AC can result in explosive gas mixtures (Di Benedetto et al., 2011). On the contrary, the CH_4 content in the upgraded biogas was not chosen as controlled variable since negligible losses are typically accounted as a result of its low aqueous solubility, while H_2S content was not considered either based on the higher H_2S removal efficiencies (REs) associated to the superior H_2S aqueous solubility compared to CO_2.

The control system operated based on the differences between the O_2 and CO_2 concentration measured in the upgraded biogas and the set point values fixed, the changes implemented in the recycling liquid flowrate being summarized in Table 1. When the O_2 content in the upgraded biogas was > 1%, the pump flow rate was decreased due to safety reasons even if the CO_2 content in the upgraded biogas was > 2% (set point value). When the O_2 content in the biomethane was <1% and CO_2 content >2%, the control system increased the flow rate of the recycling liquid pump in order to

enhance the CO_2 gas-liquid mass transfer. Finally, when the O_2 content in the biomethane was < 1% and CO_2 content < 2 %, thus complying with the standard values, the flow rate of the recycling liquid pump was also decreased in order to save energy.

Table 1. Variations in the recycling liquid flowrate as a function of the differences between the concentrations of CO_2 and O_2 in the biomethane and the set point values (ΔCO_2 and ΔO_2, respectively).

ΔO_2	**ΔCO_2**	**Power pump variation (%)**	**Liquid flow rate variation (L h^{-1})**
≤0	[(-2)-(-1)]	-6	-45.2
	[(-1)-(-0.5)]	-4	-30.1
	[(-0.5)-0]	-2	-15.1
	[0-0.5]	5	37.6
	[0.5-1]	10	75.3
	[1-5]	15	112.9
	[5-10]	20	150.5
	[10-20]	25	188.2
	>20	30	225.8
[0-0.5]	-	-5	-37.6
[0.5-1]		-10	-75.3
[1-5]		-15	-112.9
>5		-20	-150.5

2.3. Validation of the control strategy

The performance of the proposed control strategy was evaluated under different pH values in the cultivation broth (9.05, 9.20, 9.35, 9.50) for 8 h when the system operated under steady state. The initial L/G ratio was 0.8 (corresponding to the lowest L/G ratio that could be reached in the demo experimental set-up).

Process response to the stepwise variations in biogas flowrate (every 1 h and 20 min) from 143 L h^{-1} to 218, 300 and 420, and back to 143 L h^{-1}, was tested under controlled and uncontrolled conditions. The inlet pH of the cultivation broth in the AC was 9.20 and the initial liquid flowrate was maintained at 327 L h^{-1} (minimum value) during the uncontrolled conditions.

Finally, the robustness of the technology towards operational failures in biogas supply and in the liquid recirculation was assessed. After process monitoring for 4 h under

steady state, the biogas compressor or the recycling liquid pump were turned off for 2 h, and subsequently switched on again followed by process monitoring for the next 4 h under controlled and uncontrolled conditions. During the robustness test, the inlet pH of the cultivation broth in the AC was 9.35, the biogas flowrate was set at 420 L h^{-1} and the initial L/G was fixed based on the minimum L/G ratio able to provide a satisfactory biomethane quality (CO_2 content ≤ 2%) under these operational conditions (L/G ≈ 1.1-1.2).

In all experiments, the composition of the upgraded biogas was measured every 20 min prior actuation of the control system.

3. Results and discussion

3.1. Evaluation of process performance under different pH in the cultivation broth

The effect of the pH of the cultivation broth on the performance of photosynthetic biogas upgrading was evaluated. The upgraded biogas composition, L/G ratios and recycling liquid pH at the outlet of the AC under uncontrolled (initial values) and controlled conditions at different pHs of the cultivation broth (9.5, 9.35, 9.2 and 9.05) are shown in Fig. 1. In this regard, a slight drop in the pH of the cultivation broth (~0.15) caused a remarkable decrease in the CO_2 gas-liquid mass transfer in the AC under uncontrolled conditions despite the high alkalinity of the cultivation broth (1907±109 mg IC L^{-1}). The CO_2 concentration in the upgraded biogas increased from 2.7±0.1 to 4.9±0.1, 9.7±0.1 and 12.0±0.0%, which corresponded to CO_2-REs of 93.4, 87.7, 77.9 and 68.5%, at a pH of 9.50, 9.35, 9.20 and 9.05, respectively, exceeding the CO_2 set point value (2%) at a L/G ratio of 0.8 (Fig. 1a). These results agreed with those reported in a pilot scale HRAP by Bahr et al. (2014), who obtained CO_2-REs < 50% at a pH of 9 and a L/G ratio of 0.4 and CO_2-REs >90% at a pH of 10. Likewise, Rodero et al. (2019) recorded CO_2 concentrations in the upgraded biogas <2% and 16% at a pH of 10 and 8.5, respectively, under similar conditions (L/G ratio of 0.5 and 1500 mg IC L^{-1} in the cultivation broth). In this context, dissolved inorganic carbon in water is a mixture of CO_2 (aq), HCO_3- and CO_3^{2-}, the dissociation constants being pka_1= 6.35 and pka_2= 10.3 at 25ºC (Lee and Pirt, 1984). In our particular study, the dissolved inorganic carbon in the liquid phase was composed of HCO_3^- (main species) and CO_3^{2-} in the range of pH tested (9.05-9.50). In this specific range, a slight increase in pH of 0.15

shifted the equilibrium towards more CO_3^{2-} formation, thus increasing the CO_2 gas-liquid concentration gradient, and consequently higher CO_2 removals were achieved.

On the other hand, a complete H_2S removal was achieved regardless of the pH of the cultivation broth as a result of its higher aqueous solubility compared to CO_2 (according to Henry's dimensionless constant) and low concentration in the inlet biogas (52±57 ppm_v of H_2S) (Sander, 1999). Moreover, since the sulfide dissociation constants are pka_1=7.04 and pka_2=11.95 at 18ºC (Smet et al., 1998), the predominant species in the liquid phase in the range of pH studied (9.05-9.50) was HS^-, thus increasing the H_2S gas-liquid concentration gradient and consequently the mass transfer. In this context, Kang et al. (2020) observed a rapid increase in the aqueous H_2S concentration at pH 10 due to the 100 times higher H_2S equilibrium aqueous concentration in comparison with that at pH 8. On the other hand, the oxidation of HS^- in the liquid phase can be chemical (supported by the high dissolved oxygen in the cultivation broth) and/or biological (by sulfur-oxidizing bacteria, i.e. Thioalbus genus) (Meier et al., 2018; Toledo-Cervantes et al., 2016). In this regard, although sulfur oxidation can result in different products (S^0, $S_2O_3^{2-}$ and SO_4^{2-}), SO_4^{2-} is typically the major end-product due to the high dissolved oxygen (up to 21.6 mg O_2 L^{-1}) and pH in the cultivation broth of algal-bacterial photobioreactors (Kang et al., 2020; Meier et al., 2018).

Consequently, the CH_4 concentration in the upgraded biogas accounted for 97.3±0.1, 95.1±0.1, 90.3±0.1 and 88.0±0.0% at a pH of 9.50, 9.35, 9.20 and 9.05, respectively, under uncontrolled conditions, while O_2 concentration in the upgraded biogas was always negligible due to the low initial L/G ratio (0.8) (Fig. 1). In this regard, Toledo-Cervantes et al. (2017) recorded a slightly higher O_2 desorption in the upgraded biogas (O_2 content ~0.8%) under counter-current operation at a L/G ratio of 0.8 (similar conditions to this study), while the O_2 content was almost zero under co-current operation.

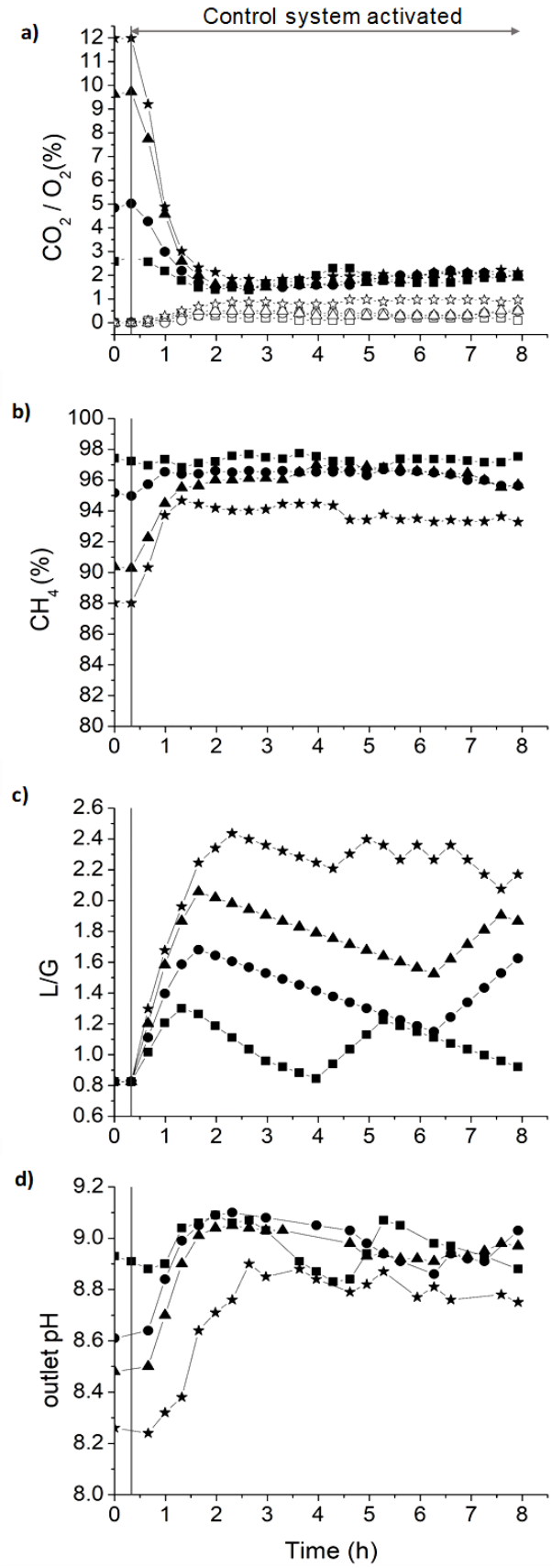

Fig. 1. Time course of a) CO_2 (solid) and O_2 (open) concentrations in the upgraded biogas, b) CH_4 concentration in the upgraded biogas, c) liquid to biogas (L/G) ratio in the absorption column and d) outlet pH of the recycling liquid in the absorption column at a pH of the cultivation broth of 9.50 (square), 9.35(circle), 9.20 (triangle) and 9.05 (star).

When the control system was initiated, the CO_2 concentration decreased to values lower than the set point (2%) after 1 h at the highest pH (9.50) and 2 h at the lowest (9.05), and remained stable afterwards (Fig. 1a). No H_2S concentration was detected in the upgraded biogas regardless of the pH. Interestingly, the O_2 concentrations in the biomethane were higher when the control was active compared to those without control as a result of the higher L/G ratios in the AC. However, these concentrations remained below the set point (O_2 concentration =1%) in most of the experiments except at a pH of 9.05, where a maximum O_2 concentration of 1% was achieved (Fig. 1b). Maximum L/G ratios of 1.3, 1.7, 2.1 and 2.4, which corresponded to liquid flowrates of 515, 681, 816 and 967 L h^{-1}, were recorded at a pH of 9.50, 9.35, 9.20 and 9.05, respectively (Fig. 1c). In fact, a lower decrease in the pH along the AC was obtained when the control system was active (0.2±0.1 vs 0.7±0.1) due to process operation at higher L/G ratios (Fig. 1d). This lower decrease in the pH at higher L/G ratios was associated to the lower mass of CO_2 transferred per recycling liquid volume (Table S1), which allowed to achieve higher CO_2-REs (Posadas et al., 2017). In this context, the limited acidification of the liquid along the AC due to the higher L/G ratios when the system was controlled resulted in higher CO_2-REs. This was mediated by the equilibrium shift from CO_2 to HCO_3^- and CO_3^{-2}, which supported higher gas-liquid CO_2 concentration gradients.

3.2. Process response to stepwise variations in biogas flowrate

The daily production of biogas might vary as a result of changes in the feedstock mass flowrate or composition and temperature in the anaerobic digester, which directly impacts on the upgrading process (Kim and Lee, 2016; Theuerl et al., 2019). Fig. 2 shows the upgraded biogas composition and liquid flowrate in the AC under controlled and uncontrolled conditions during the stepwise variations in biogas flowrate from 143 L h^{-1} to 218, 300 and 420, and back to 143 L h^{-1}.

The CO_2 concentration in the upgraded biogas increased from 2.5 to 14.1%, when the biogas flowrate was stepwise increased from 143 to 218, 300 and 420 L h^{-1} under uncontrolled conditions (at a constant liquid flowrate of 327 L h^{-1}), which corresponded to a decrease in the L/G ratio from 2.3 to 0.8. These results were in accordance with Marín et al. (2019), who reported a decrease in the CO_2 content from 9.6% to negligible values when increasing the L/G ratio from 0.5 to 2.0. Subsequently, when the biogas flowrate was stepwise decreased from 420 to 300 L h^{-1}, the CO_2 concentration slightly increased up to 16.1% as a result of the previous acidification of the liquid remaining in

the AC. Then, the concentration of CO_2 gradually decreased to 6.0% at the lowest biogas flowrate of 143 L h^{-1} (Fig. 2a). The O_2 and H_2S concentrations in the upgraded biogas were negligible in the absence of control strategy, while CH_4 concentration was correlated to CO_2 removal, with a maximum concentration of 97.6% at 143 L h^{-1} (at the beginning of the assay) and a minimum CH_4 concentration in the upgraded biogas of 83.9% at 300 L h^{-1} (after the decrease from 420 L h^{-1}) (Fig. 2b). Overall, the system was not able to achieve a biomethane quality complying with most international standards (CO_2 content ≤ 2% and CH_4 content ≥ 90%) without control system.

Biomethane quality improved significantly when the control system was active. Indeed, the CO_2 concentration recorded in the upgraded biogas reached a maximum of 2.4% (~6.7 times lower than that without control) and remained almost constant at ~2% regardless the stepwise variations in biogas flowrate from 143 L h^{-1} to 218, 300 and 420, and back to 143 L h^{-1} (Fig. 2a). A complete H_2S removal was achieved, while low O_2 concentrations in the biomethane (≤ 0.5 %) were recorded even at the maximum L/G ratio of 4.9 (corresponding to a liquid flowrate of 703 L h^{-1}) (Fig. 2c). These high L/G ratios occurred during the stepwise decrease in the biogas flowrate, since the liquid flowrates imposed by the control system were still high due to the culture broth acidification caused by the previous biogas flowrates. In this context, the lower O_2 desorption recorded at higher L/G ratios compared to that reported in section 3.1, where the O_2 concentration in the biomethane was 1% at a pH of 9.05 and a L/G ratio of 2.3, could be attributed to the higher liquid flowrate reached in the previous section (967 L h^{-1}) and the lower biogas flowrate (143 or 218 L h^{-1}) in the present experiment, which supported a lower turbulence in the AC and a lower O_2 gas-liquid mass transfer in this unit. In this context, turbulence in the AC impacts on the average bubble size, which itself is inversely proportional to both components of the overall mass transfer coefficient (kla): the specific area (a) and the liquid transport coefficient (kl) (Bordel et al., 2008). Finally, it should be stressed that the CH_4 concentration in the upgraded biogas was >95.5% during the complete experimental period under controlled conditions (Fig. 2b). In brief, the control strategy implemented was effective to cope with variations in the biogas flowrate over time.

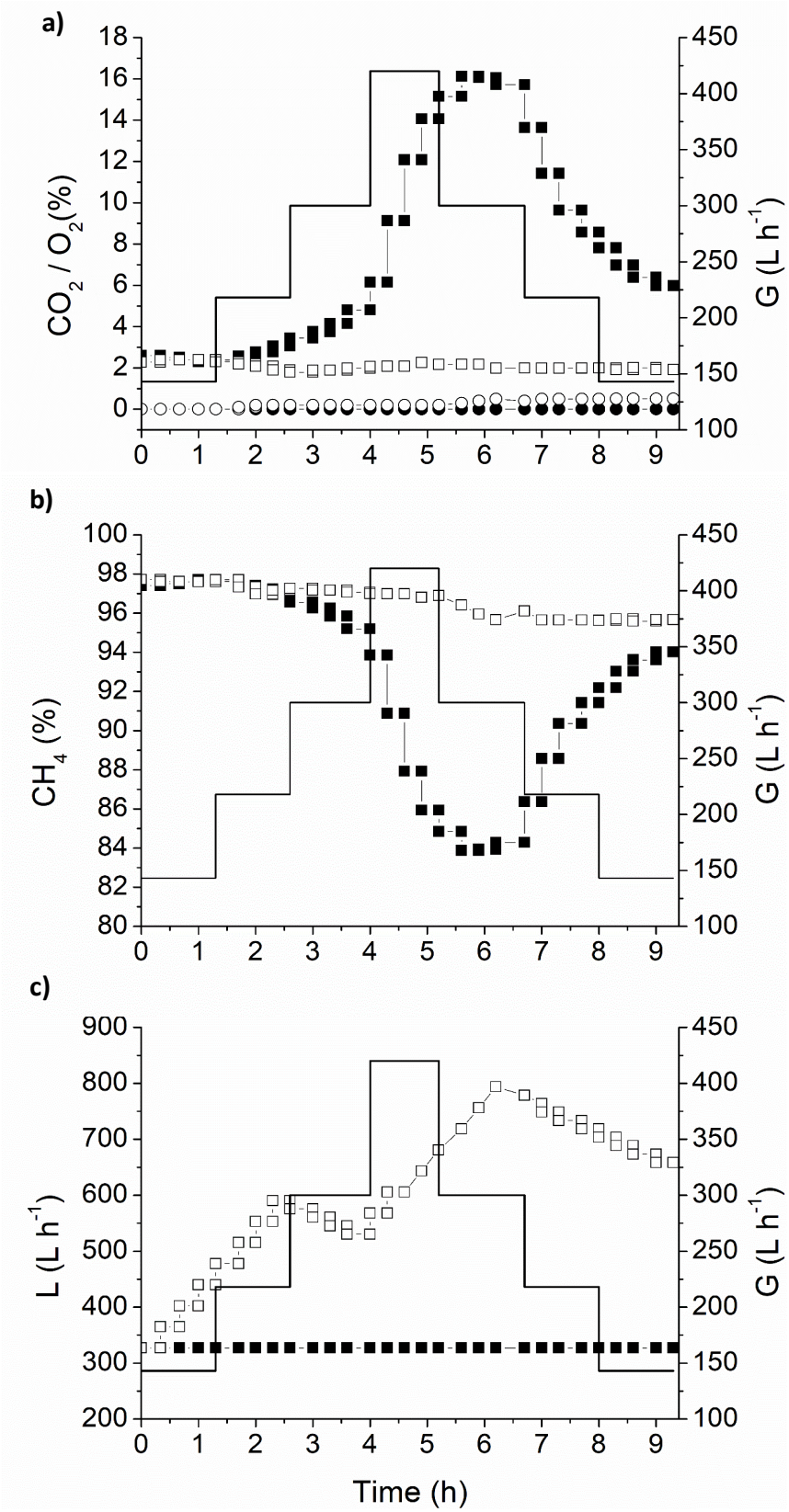

Fig. 2. Time course of a) CO_2 (square) and O_2 (circle) concentrations in the upgraded biogas, b) CH_4 concentration in the upgraded biogas and c) liquid flowrate (L) under controlled (open) and uncontrolled (solid) conditions during the stepwise variation in biogas flowrate (G) (continuous line).

3.3. Robustness under operational failures in biogas supply and in the liquid recirculation

Operational failures typically occur in biogas upgrading plants at full scale, which impacts on biomethane quality during the failure and/or afterwards when the system is restored. This requires the evaluation of the control system performance under the most relevant equipment failures in photosynthetic biogas upgrading (stoppage of biogas supply or liquid recirculation). The upgraded biogas composition and liquid flowrate in the AC under controlled and uncontrolled conditions during a 2h failure in biogas supply or liquid recirculation are shown in Fig. 3 and 4, respectively.

Under uncontrolled conditions at a L/G ratio of 1.1, the CO_2 concentration in the upgraded biogas accounted for 1.8±0.1% during the initial hours of the experiment assessing the robustness of the technology against a failure in biogas supply. The concentration of CO_2 remained constant at 1.9% for the next 2 h without biogas supply (Fig. 3a), which could be attributed to the biomethane accumulated in an open to atmosphere gasometer located immediately after the biogas analyzer. Interestingly, the CH_4 concentration was negatively impacted by the biogas compressor failure, decreasing from 98.1 to 62.6% after 2 h without biogas supply (Fig. 3b). This decrease can be explained by the entrance of air in the system, which was confirmed by the increase in O_2 concentration up to 7.5% after 2 h (Fig. 3a). When biogas supply was re-started, the O_2 concentration rapidly decreased to 0.3% within 20 minutes, with an associated increase in CH_4 concentration up to 96.8 %, CO_2 concentrations ~1.8% and no H_2S detected (Fig. 3a, b). This rapid increase in CH_4 content was mediated by the high biogas flowrate used during this experiment (420 L h^{-1}), which flushed the air out of the system. However, the CO_2 concentration slightly increased to ~2.2% following 1.5 h from the restoration of biogas supply (due to a slight decrease in the pH of the cultivation broth) and remained constant afterwards.

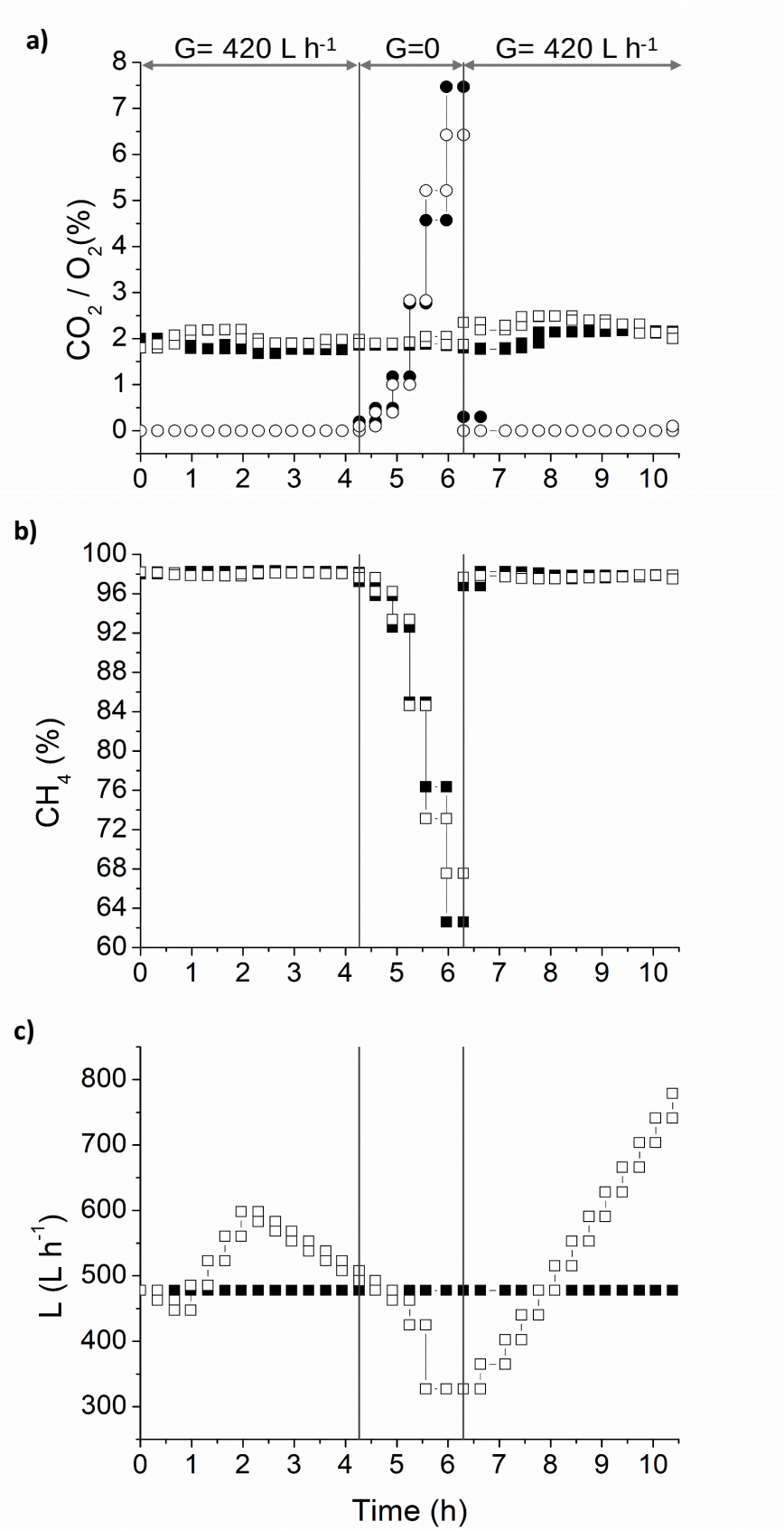

Fig. 3. Time course of a) CO_2 (square) and O_2 (circle) concentrations in the upgraded biogas, b) CH_4 concentration in the upgraded biogas and c) liquid flowrate (L) under controlled (open) and uncontrolled (solid) conditions during a failure in biogas supply (G).

When the control system was initiated, the liquid flowrate fluctuated between 448 and 598 L h^{-1} during the first hours of experiment in order to maintain biomethane quality under optimal conditions in terms of energy consumption (Fig. 3a, c). The CO_2 content in the absence of biogas supply remained constant at ~ 1.9%, while an increase in the O_2 concentration from 0 to 6.4% was recorded as a result of air entrance, similar to that observed without control system (Fig. 3a). Thus, the control system decreased the liquid flowrate down to the minimum value (327 L h^{-1}) in order to prevent a high O_2 content in the upgraded biogas. In this context, when biogas supply was restarted, CO_2 concentration in the upgraded biogas increased up to 2.5% as a result of the low liquid flowrate. Nevertheless, the system was able to decrease the CO_2 concentration to 2% by the end of the experiment by imposing a liquid flowrate of 779 L h^{-1} (Fig. 3a, c). The CH_4 concentration in the biomethane decreased from 98.0 to 67.5% in the absence of biogas supply, increasing to 97.7% within only 20 min after the resumption of biogas supply (Fig. 3b). No H_2S was detected in the upgraded biogas along the experiment under controlled conditions. Overall, similar results were obtained under controlled and uncontrolled conditions, the system without control being even more effective when biogas supply was restarted. However, in case of an eventual increase in the CO_2 content resulting from any variation in the cultivation broth, the system would not be able to recover the initial CO_2 concentration without control.

CO_2 content in the upgraded biogas remained constant at 1.9±0.1% during the first hours under uncontrolled conditions at a L/G of 1.2 in the experiment assessing the robustness of the technology against a shutdown in the liquid supply to the AC. When the recirculating liquid pump was turned off, CO_2 concentration in the upgraded biogas rapidly increased up to 28.9% within 2 h, which almost matched the CO_2 concentration of the raw biogas (31.5±1.1%). This poor CO_2-RE was due to the acidification and CO_2 saturation of the liquid present in the biogas AC. However, the CO_2 concentration in the upgraded biogas rapidly decreased when the liquid pump was turned on since the liquid retention time in the AC was only 17.5 min under the working liquid flowrate (515 L h^{-1}). Unfortunately, the system was not able to recover the initial biomethane quality, with CO_2 concentrations of 2.3% after approximately 2.5 h from liquid supply restoration (Fig. 4a). On the other hand, the CH_4 content in the upgraded biogas decreased from 97.9 to 71.1% and increased up to 97.7% when the liquid pump was restarted (Fig. 4b). Despite the acidification of the scrubbing solution during the period without liquid

renewal in the AC, negligible H_2S concentrations (1 ppm_v) were detected as a result of its low concentration in the raw biogas. Finally, no significant O_2 concentrations (<0.2%) were recorded in the upgraded biogas along this experiment.

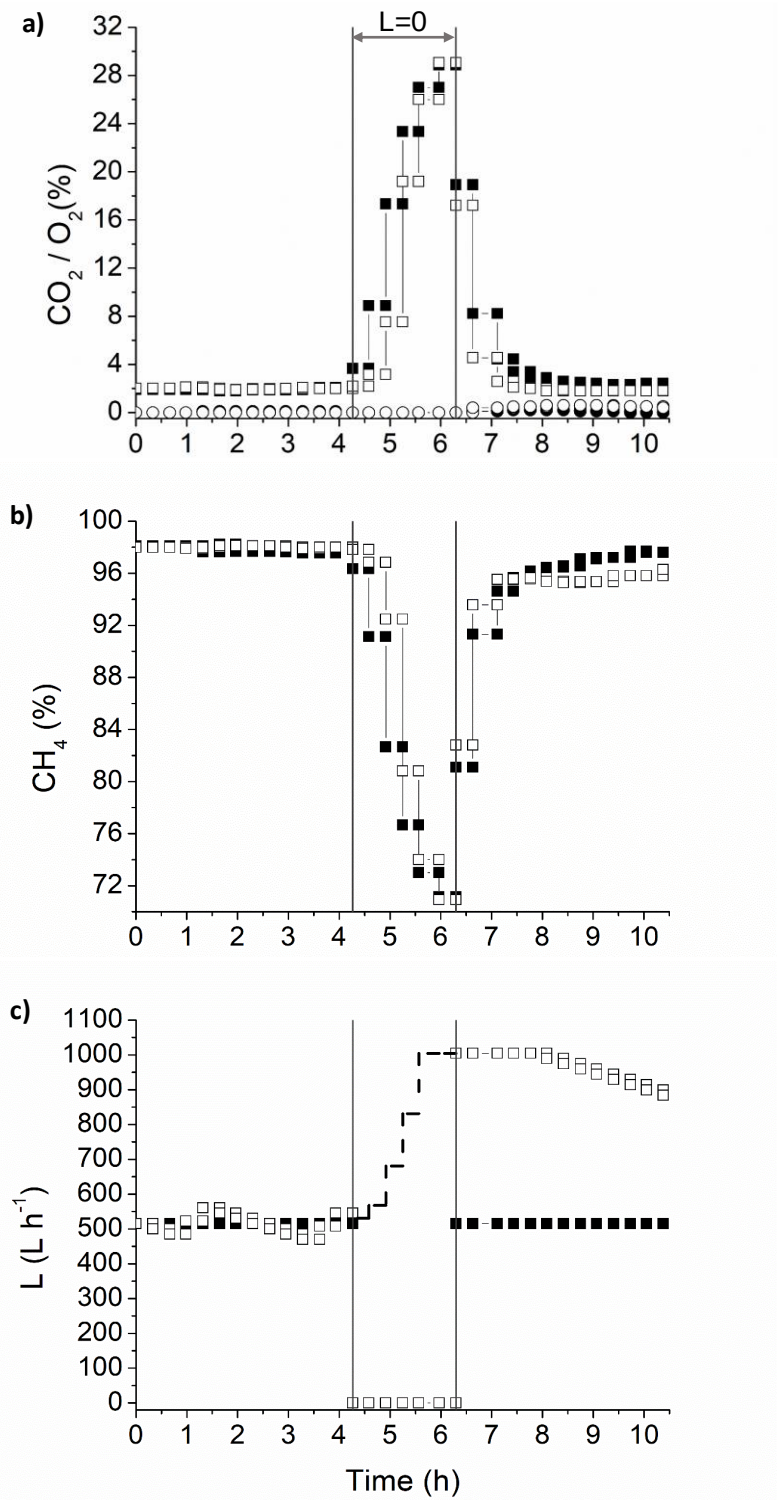

Fig. 4. Time course of a) CO_2 (square) and O_2 (circle) concentrations in the upgraded biogas, b) CH_4 concentration in the upgraded biogas and c) liquid flowrate (L) under controlled (open) and uncontrolled (solid) conditions during a failure in liquid recirculation. The control system unit changes when the liquid pump was off are represented by a dashed line (c).

When the control system was active, minor variations in the liquid flowrate were recorded (470-560 L h^{-1}) and the CO_2 content remained below 2% (Fig. 4a, c). When the liquid recirculation was stopped, the CO_2 concentration in the upgraded biogas increased up to 29.1%, but no H_2S was detected as under uncontrolled conditions (Fig. 4a). The control system sent control actions of increasing the liquid flowrate (CO_2 measured> CO_2 set point and $O_2 \approx 0$) during the period with no liquid supply since it was not able to detect the liquid pump failure. Therefore, when the liquid pump was switched on, the liquid flowrate imposed by the control system corresponded to the maximum pump flowrate (~1000 L h^{-1}). This entailed a decrease in the CO_2 content of the upgraded biogas faster than under uncontrolled conditions due to the higher L/G ratio (2.4 *vs* 1.2) (Fig. 4c). However, the decrease in the CO_2 content could have been even faster if higher pumping capacity would be available. On the other hand, the O_2 content in the upgraded biogas increased when the liquid pump was turned on as a result of the high liquid flowrate, but remained always below 1%. Finally, the CH_4 content in the upgraded biogas decreased from 98.0 to 70.9% due to the negligible CO_2-REs in the absence of liquid recirculation. Nevertheless, CH_4 content rapidly increased up to 95.8% when the liquid supply was restored although this value was lower compared to process operation without control system. This decrease was mediated by the higher O_2 and N_2 desorption from the recycling liquid to the biomethane as a result of the higher recycling liquid flowrate. Overall, the control system was able to provide a satisfactory biomethane quality in the event of a liquid supply stoppage, while in the absence of control system the CO_2 concentration remained >2% after liquid supply restoration.

4. Conclusions

The control system based on changes in the recycling liquid flowrate was able to meet the target biomethane quality (CO_2<2% and O_2<1%) regardless of the pH and biogas flowrate. Despite the poor robustness of this technology against failures in biogas and liquid supply was confirmed, the control system restored the biomethane quality satisfactorily after the event of a stoppage in biogas supply and liquid recirculation. This control strategy validated in an outdoors semi-industrial scale photobioreactor would overcome the negative effects of environmental variations or operational failures on photosynthetic biogas upgrading performance, ensuring a consistent biomethane quality.

Acknowledgements

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Supplementary Material

Performance evaluation of a control strategy for photosynthetic biogas upgrading in a semi-industrial scale photobioreactor

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Content:

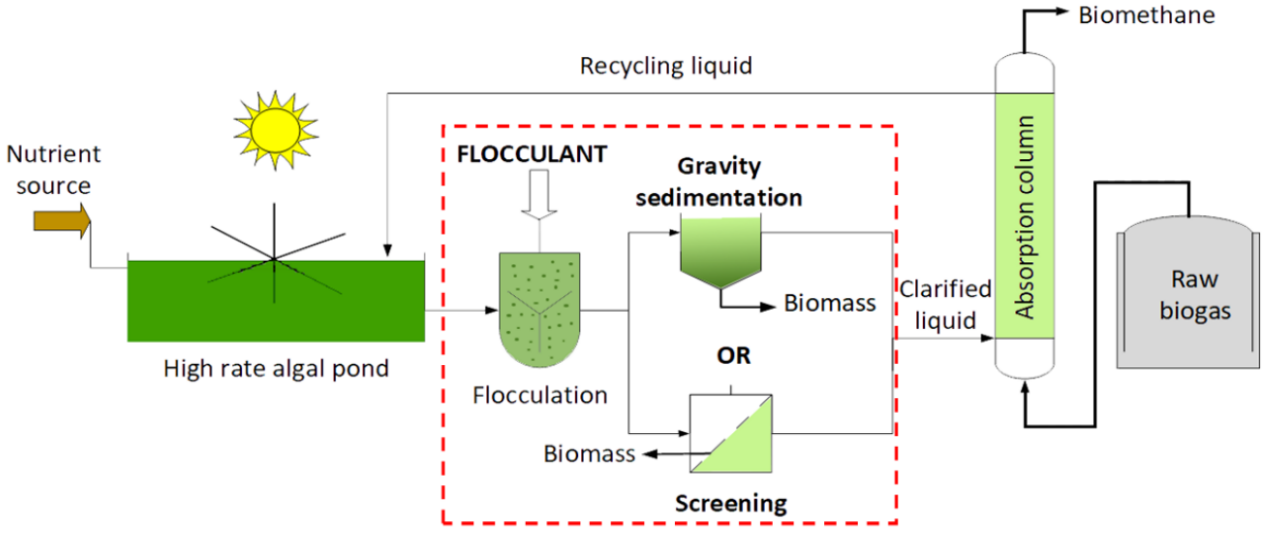
Table S1. Liquid flowrate and CO_2 mass transferred per volume of recycling liquid during the experiment at different pHs.

pH =9.50		**pH =9.35**		**pH =9.20**		**pH =9.05**	
Liquid flowrate ($L\ h^{-1}$)	**CO_2 transferred per volume of liquid ($mg\ L^{-1}$)**	**Liquid flowrate ($L\ h^{-1}$)**	**CO_2 transferred per volume of liquid ($mg\ L^{-1}$)**	**Liquid flowrate ($L\ h^{-1}$)**	**CO_2 transferred per volume of liquid ($mg\ L^{-1}$)**	**Liquid flowrate ($L\ h^{-1}$)**	**CO_2 transferred per volume of liquid ($mg\ L^{-1}$)**
327	629	327	587	327	569	327	459
327	626	327	584	327	567	327	459
402	511	440	443	478	413	515	325
478	435	553	365	628	341	666	289
515	407	628	328	741	303	779	261
500	422	666	314	816	279	892	232
470	451	651	322	801	287	929	223
440	480	636	329	786	292	967	216
410	517	621	338	771	299	952	220
380	555	606	346	756	305	937	224
365	577	591	355	741	310	922	227
350	600	575	364	726	317	907	231
335	624	560	374	711	323	892	235
372	556	545	384	696	330	877	238
410	505	530	395	681	336	914	228
448	466	515	404	666	345	952	219
485	432	500	415	651	352	937	222
470	447	485	427	636	360	899	232
455	462	470	441	621	367	937	223
440	478	455	454	606	376	899	231
425	495	493	418	643	353	937	222
410	512	530	390	681	334	899	231
395	530	568	364	718	317	861	241
380	551	606	342	756	301	824	251
365	572	643	322	741	308	861	241

Chapter 8

Harvesting microalgal-bacterial biomass from biogas upgrading process and evaluating the impact of flocculants on their growth during repeated recycling of the spent medium

Rodero, M. del R., Muñoz, R., Lebrero, R., Verfaillie, A., Blockx, J., Thielemans, W., Muylaert, K., Praveenkumar, R., 2020. Algal Res. 48, 101915. doi:10.1016/j.algal.2020.101915



Harvesting microalgal-bacterial biomass from biogas upgrading process and evaluating the impact of flocculants on their growth during repeated recycling of the spent medium

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ABSTRACT

Microalgal-bacterial consortium can be used to upgrade biogas by removing CO_2 and H_2S. Photosynthetic biogas upgrading requires harvesting microalgal-bacterial biomass in order to use the biomass-free cultivation medium as scrubbing liquid in the absorption column. In this study, the efficiency of different flocculants (Zetag 8125, cationically modified cellulose nanocrystals, Tanfloc, chitosan, and $FeCl_3$) to harvest microalgal-bacterial biomass used for biogas upgrading in alkaline medium (inorganic carbon concentration up to 1800 mg L^{-1} and a pH ~10) was evaluated. Zetag and cationic cellulose nanocrystals resulted in maximum flocculation efficiencies of 95% (optimal dose 30 mg g^{-1}) and 93% (optimal dose 20 mg g^{-1}), respectively. Low flocculation was observed with other flocculants at doses as high as 200 mg g^{-1}, which can be ascribed to the high pH of the alkaline medium. Zetag and cationic cellulose nanocrystals were selected for harvesting the biomass during semi-continuous cultivation of the microalgal consortium. Both Zetag and cationic cellulose nanocrystals were effective in flocculating the biomass with efficiencies of over 90% during five successive harvesting cycles. Gravity settling of the flocs formed by Zetag and cationic cellulose nanocrystals resulted in low biomass concentration factors of 7.7 and 2.0, respectively. Screening of flocs using a nylon mesh screen (pore size of 180 μm) resulted in a biomass concentration factor as high as 19.8. Zetag and cationic cellulose nanocrystals could be useful in harvesting biomass under high alkaline conditions without detrimental effects on biomass growth.

Keywords: Microalgae; Harvesting; Flocculation; Cellulose nanocrystals; Zetag; Screening

1. Introduction

Biogas from the anaerobic digestion of organic waste or wastewater constitutes a promising renewable energy vector able to reduce our current dependence on fossil fuels due to its high CH_4 content (40-75%) [1]. In this context, the removal of biogas pollutants, mainly CO_2 and H_2S, is a mandatory step for its use as a natural gas substitute [2]. Photosynthetic biogas upgrading in high-rate algal ponds coupled with an external absorption column has recently emerged as a low cost (energy consumption of 0.08 kW-h $(Nm^3_{\text{ treated biogas}})^{-1}$) and environmentally friendly (CO_2 emissions of 21 g-CO_2 $(Nm^3_{\text{ treated biogas}})^{-1}$) alternative to conventional physical-chemical technologies to remove CO_2 and H_2S from biogas (energy consumption and CO_2 emissions of 0.30 kWh and 944 g-CO_2 to obtain 1 Nm^3 of treated biogas, respectively, for an activated carbon filter combined with a water scrubbing) [3]. Maintaining a high alkalinity (inorganic carbon concentration >1500 mg L^{-1}) and pH ~10 of the cultivation medium is essential to increase the mass transfer of acidic gases like CO_2 and H_2S from the biogas to the cultivation medium [4]. Hence, the use of alkaliphilic microalgal-bacterial consortia able to withstand high inorganic carbon concentrations is essential to efficiently remove CO_2 and H_2S from the cultivation medium in high-rate algal ponds [5]. The biogas upgrading process is based on the use of part of the biomass-free cultivation medium as scrubbing liquid in the absorption column. In this sense, separating the microalgal-bacterial biomass generated in high-rate algal ponds from the scrubbing liquid constitutes a critical step. It also allows for control over microalgal productivity under operation with no effluent as a consequence of evaporation losses of water when using digestate as nutrient source (due to its high nutrient concentration, which consequently requires low digestate flowrates to sustain algal-bacterial growth)[6].

Several microalgae harvesting methods such as centrifugation, flotation, sedimentation, or filtration have been reported [7]. However, due to low biomass concentration of microalgae in high-rate algal ponds (0.2-1.2 g L^{-1}) and their small cell size (typically in micrometers), some of these technologies do not achieve an efficient solid-liquid separation or they are limited by high-energy requirements with associated increases in operational costs [8,9]. In this regard, flocculation followed by a solid-liquid separation step, such as gravity sedimentation or screening, is considered a rapid and cost-effective alternative for a large-scale harvesting of microalgal biomass [10]. During flocculation, the addition of chemicals leads to the aggregation of microalgal cells forming large

flocs [11]. Flocculation can be induced by neutralizing the surface charge of the cells (charge neutralization), by partially reversing the charge of the particle surface, resulting in the connection of particles through patches with opposite charge (electrostatic patch), by precipitation caused by an aggregating polymer network that entangles microalgal cells (sweeping mechanism), or by forming bridges between individual particles (bridging) [12,13].

The optimal dose of the flocculants depends on the characteristics of the microalgal species (i.e. cell size, culture age, and cell wall composition) and the flocculant (e.g. charge, rigidity, and morphology) [14]. Inorganic salts, such as $FeCl_3$, which induce flocculation via charge neutralization, have been widely used as flocculants due to their low cost, in spite of needing higher dose compared to other flocculants [15,16]. Organic polymers such as Zetag, a synthetic copolymer of acrylamide and quaternized cationic monomers, which are able to interact with microalgal cells by charge neutralization and bridging, have been successfully applied in the flocculation of various microalgae [17,18].

Flocculants based on natural biopolymers are attracting interest as flocculants due to their biodegradability. Chitosan from chitin waste is a non-toxic and inexpensive biopolymer composed of linear poly-amino-saccharide chains that can agglomerate individual cells through different mechanisms such as charge neutralization, bridging, sweeping, and adsorption [19–21]. Tanfloc is a commercial biopolymer based on tannins extracted from bark of *Acacia mearnsii* that has also been used as a flocculant for microalgae [18,22]. More recently, cationically modified cellulose nanocrystals (CNCs) have been introduced as a flocculant for microalgae [23–26]. CNCs have a high aspect ratio and high external surface area (~300 m^2 g^{-1}), which is favorable for flocculation. Moreover, they can be readily modified by addition of a wide range of polymer matrices to obtain a flocculant with desired surface characteristics [27,28].

The pH of the culture medium is one of the crucial factors for the performance of the flocculants. Many flocculants get protonated and become cationic only at low pH (<7) [29]. In an alkaline medium, flocculants that carry a pH-independent cationic charge should have a superior performance. Many polymer flocculants experience coiling in high ionic strength conditions and are expected to perform poorly in a medium with a high inorganic carbon concentration [30,31]. Hence, the selection of a flocculant that functions at high pH and at high inorganic carbon concentration is essential for

photosynthetic biogas upgrading. Another important feature while applying flocculants in biogas upgrading systems is to obtain a biomass-free medium that can be repeatedly recycled without any detrimental effect on the growth of microalgae and bacteria. Recycling of the spent medium from the absorption column to the photobioreactor is essential for the subsequent removal of CO_2 and H_2S from the medium. While CO_2 will be consumed by microalgae, H_2S will be oxidized to sulphate by sulphur oxidizing bacteria using the oxygen that is generated photosynthetically [32]. In this regard, it is important that accumulation of the flocculant and/or algal organic matter in the recycled culture medium should not lead to microalgal-bacterial growth inhibition [33,34]. Furthermore, the flocculant needs to be versatile in harvesting altogether different microalgal species present in the consortium. Otherwise, those species of microalgae that did not flocculate would eventually alter the microalgal community structure and ultimately make the flocculation process inefficient. So far, no studies have focused on the selection of a suitable flocculant and its dose for efficient use in a repeated recycling of cultivation medium, in spite of the crucial role of this separation step in photosynthetic biogas upgrading.

The aim of this study was to optimize harvesting of a microalgal-bacterial consortium using flocculation, followed by a solid-liquid separation for a photosynthetic biogas upgrading process which requires working under high pH (~10) and alkalinity (inorganic carbon concentration up to 1800 mg L^{-1}), and to evaluate the effect of flocculants on the biomass while recycling the culture medium. For this purpose, different flocculants such as, Zetag® 8125, cationic CNCs, Tanfloc, chitosan, and $FeCl_3$ were tested. Furthermore, the recyclability of the medium after flocculation for the effective flocculants (Zetag and cationic CNCs) was evaluated in a semi-continuous cultivation system. Finally, the feasibility of using screening instead of gravity settling to separate biomass flocs from the culture medium was also assessed.

2. Materials and methods

2.1.Cultivation of microalgal-bacterial consortium

Microalgal-bacterial consortium was obtained from an indoor high-rate algal pond used for biogas upgrading using a high alkalinity synthetic medium as nutrient source located at the Department of Chemical Engineering and Environmental Technology at University of Valladolid. The consortium was grown in 2 L bottles (diameter: 136 mm,

working volume: 1.5 L) as fed-batch cultures in a synthetic medium composed of (g L^{-1}): 7.60 $NaHCO_3$, 3.70 Na_2CO_3, 0.58 K_2HPO_4, 1.91 NH_4Cl, 0.10 $MgSO_4 \cdot 7H_2O$, 0.02 $CaCl_2 \cdot 2H_2O$ and 1 mL of a trace metal solution prepared according to the Wright's cryptophyte medium [35]. The cultivation medium was maintained at pH ~10 and fed with 25 mL of fresh medium every day, based on the data on the hydraulic retention time used in the high rate algal pond for biogas upgrading [36]. The flasks were aerated by bubbling with 0.2-µm filtered air and mixed using magnetic stirrers. Cultures were continuously illuminated from front and backside of the flask, each at an intensity of ~100 µmol m^{-2} s^{-1} and maintained at 24 ºC in a temperature-controlled room.

2.2. Selection of optimal flocculants for use in alkaline and high pH conditions

Flocculation efficiencies of five flocculants: Zetag® 8125 (BASF, Germany, hereinafter referred as Zetag), in-house developed CNCs grafted with methylimidazolium cationic group (MIM-*g*-CNCs) [25], $FeCl_3 \cdot 6H_2O$ (Chem-lab, >99%), Tanfloc® SG (Tanac, Brazil), and chitosan (Sigma-Aldrich 417963) were tested on the microalgal-bacterial consortium using standard jar tests. For each flocculant a stock solution of 5 g L^{-1} was prepared in distilled water. The stock solution of chitosan (5 g L^{-1}) was prepared in a 0.04 M HCl solution due to its slow dissolution in distilled water [20].

To evaluate harvesting of microalgae-bacterial biomass using different flocculants, conditions for the jar test such as initial stirring speed (300 – 900 rpm), stirring time (5 – 30 min), floc settling time (15 – 120 min), and biomass concentration (0.2 – 2 g L^{-1}) were initially optimized with 30 mg g^{-1} of Zetag or MIM-*g*-CNCs in order to achieve optimal flocculation efficiency and biomass concentration factor (Supplementary material, Fig. S1).

Dose-response curves for the flocculants were determined by adding different concentrations of flocculants (ranging from 0 to 200 mg g^{-1}) to 50 mL of microalgae-bacteria suspension (~1 g L^{-1} TSS) while vigorously mixing at 700 rpm with a magnetic stirrer. Following the addition of flocculants, the suspension was gently mixed at 200 rpm for 5 min to promote flocculation. After this, the suspension was decanted in 50 mL plastic tubes and the flocs were allowed to settle for 60 min before measuring the volume and the optical density (750 nm) of the supernatant (Genesis 10S UV−Vis; Thermo Fisher, US). The flocculation efficiency (η_a) was calculated based on measurement of the optical density before flocculants addition (OD_i) and of the supernatant after settling (OD_f) according to the following equation:

$$\eta_a = \frac{OD_i - OD_f}{OD_i} \qquad (1)$$

In addition, the biomass concentration factor was calculated as:

$$CF = \frac{C_f}{C_i} \qquad (2)$$

where C_i and C_f were the initial biomass concentration before addition of flocculants and final biomass concentration in the volume containing the flocculated microalgae, respectively. The jar tests were carried out in duplicate and the results were represented as the average values along with their corresponding standard deviation.

2.3. Repeated recycling of spent medium

Based on the performance of the flocculants, Zetag and MIM-*g*-CNCs were chosen for experiments with repeated recycling of the spent medium in order to check the effectiveness of the flocculants in a semi-continuous cultivation system. In these experiments, three 2 L bottles (working volume 1.5 L) with synthetic medium were inoculated with the microalgal-bacterial consortium (initial biomass concentration of 0.2 g L^{-1}) and incubated under similar conditions as described in section 2.1. Following 4 days of incubation, 500 mL of the culture from each bottle were harvested either by centrifugation or by Zetag or MIM-*g*-CNCs-based flocculation, and the spent medium was recycled to the culture bottles. The working volume of the cultures was maintained at 1.5 L by addition of fresh medium (NH_4^+ concentration of 100 mg L^{-1} to avoid ammonia inhibition) after harvesting in order to compensate losses in the spent medium. The harvesting of the control cultures was performed by centrifugation at 6000 rpm for 10 min following 30 min settling to test autoflocculation. For Zetag or MIM-*g*-CNCs - based flocculation, the suspensions in a beaker were mixed intensively (250 rpm) with an overhead stirrer for 1 min following the addition of the flocculant. Then, the suspensions were gently mixed (50 rpm) for another 20 min, after which they were allowed to settle for 30 min in a 500 mL Imhoff cone. The recycling experiments were repeated for 5 cycles during 14 days with doses for Zetag and MIM-*g*-CNCs ranging from 25 – 49 and 20 – 40 mg L^{-1}, respectively.

The specific growth rate (μ) was calculated as:

$$\mu = \frac{\ln(C_2/C_1)}{t_2 - t_1} \qquad (3)$$

where c_1 and c_2 were the biomass concentration at times t_1 and t_2.

The biomass concentration was measured as total suspended solids (TSS; g L^{-1}). TSS was determined gravimetrically based on GF/C filtration (Whatman, UK) and drying of biomass at 105 °C overnight after washing them 2 – 3 times with distilled water in order to remove the inorganic salt residue [37]. A linear correlation of optical density values of the culture at 750 nm against TSS (TSS g L^{-1} = 0.7234 × $OD_{750\ nm}$ – 0.0699) was obtained. The pH of the culture medium was monitored every day (Consort C1010; Consort bvba, Belgium) and adjusted to ~10 before the harvesting by adding the necessary volume of 2 M HCl solution. ζ-Potential of the cultivation medium was measured (NanoBrook Omni; Brookhaven Instruments, US) in triplicate before and after flocculation to monitor the flocculant accumulation in the spent medium and the results were represented as the average values along with their corresponding standard deviation. The inorganic carbon concentration was measured before flocculation using a carbonate hardness test (Merck Millipore, Germany).

2.4. Separation of flocs by gravity sedimentation and screening

Screening using a nylon mesh screen with pore size of 180 µm (Elko filtering Co., Switzerland) was evaluated for solid-liquid separation following flocculation to increase the concentration factor. Biomass was flocculated with either Zetag (20 mg g^{-1}) or MIM-*g*-CNCs (40 mg g^{-1}) and allowed to settle for 30 min. Following settling, the entire volume of the suspension was screened through the nylon mesh screen. The flocculation efficiency and the concentration factor were calculated as described in section 2.2. These experiments were carried out in duplicate and the results were represented as the average values along with their corresponding standard deviation.

3. Results and discussion

3.1. Flocculation of microalgal-bacterial biomass from fed-batch cultures

The microalgal-bacterial consortium was mainly composed of *Chlorella* sp., *Oscillatoria* spp., and uncharacterized bacterial species. Microscopic observation at different time points of fed-batch cultivation confirmed the stable composition of the microalgal consortium.

Among the five different flocculants tested, Zetag and MIM-*g*-CNCs resulted in efficient flocculation of the microalgal-bacterial consortium. While Zetag triggered a maximum flocculation efficiency of 95% with a dose of 30 mg g^{-1} (g flocculant g^{-1} dry

matter biomass concentration), MIM-*g*-CNCs resulted in a flocculation efficiency of 93% with 20 mg g^{-1} (Fig. 1). Both are cationic polymeric flocculants carrying respectively quaternary ammonium and methyl imidazolium groups, i.e. cationic charges that are stable over a very wide pH range. Other synthetic cationic polymers have been reported for harvesting marine microalgae, such as Zetag 7557 and Synthofloc 5080H to harvest *Phaeodactylum tricornutum* and *Neochloris oleoabundans* at a pH 7.5 [17], and Magnafloc to harvest *Chaetoceros calcitrans* at a pH 10.2 [38]. With freshwater microalgae *C. vulgaris*, flocculation efficiency of 99% was reported with Zetag 8125 with a dose of 6.4 mg g^{-1}, whereas, with marine microalgae *Nannochloropsis oculata* a flocculation efficiency of ~44% with a dose of 155 mg g^{-1} was reported [18]. In spite of the high pH (~10) and high inorganic carbon concentration (~1800 mg L^{-1}), a superior flocculation efficiency (95% with 30 mg g^{-1}) was achieved with Zetag 8125 in this study when compared to the flocculation of *Nannochloropsis oculata*. This could be attributed to the relatively low ion concentration in the alkaline medium used in this study compared to the marine culture medium.

In this study, in addition to Zetag, the efficiency of the methyl imidazolium-modified natural cellulose in the form of ribbon-like nanocrystals to harvest microalgal-bacterial consortium at high pH (~10) and inorganic carbon concentrations (up to 1800 mg L^{-1}) was demonstrated. Verfaillie *et al.* [26] reported a slight decrease in the flocculation efficiency (from 96% to 87%) with the increase of salinity from 0 to 50 g L^{-1} when using 20 mg L^{-1} of cationic CNCs to harvest *Nannochloropsis oculata.* With freshwater microalgae *C. vulgaris*, Blockx *et al.* [25] reported flocculation efficiencies >80% with 50 mg L^{-1} cationic CNCs at a pH 6 and a biomass concentration of 0.28 g L^{-1}. Reportedly, cationically modified CNCs are efficient and versatile in the sense that they could be used to flocculate microalgae grown under a wide range of cultivation conditions due to their pH independent charge, crystalline nature that provides rigidity to avoid coiling of the polymer under high ionic strength medium, and finally, a high surface cationic charge density that results in high flocculation efficiency at low doses [25,26].

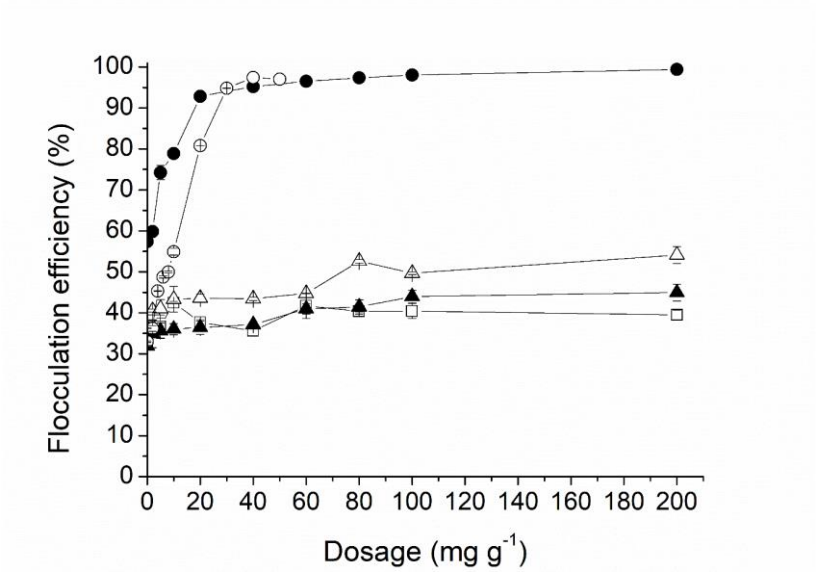

Fig.1. Flocculation dose-response curves (average values and standard deviation; n=2) of Zetag (○), cationic cellulose nanocrystals (●), $FeCl_3$ (Δ), Tanfloc (▲) and Chitosan (□).

Other flocculants such as $FeCl_3$, Tanfloc, and chitosan resulted in low flocculation efficiencies (maximum values of 54±2, 45±2 and 43±0%, respectively) for doses up to 200 mg g^{-1} (Fig. 1). When compared to organic polymers, inorganic salts such as ferric chloride often requires higher doses to promote flocculation [39]. However, doses higher than 200 mg g^{-1} could result in toxicity of the medium and, moreover, the presence of residual metal ions in the harvested biomass could pose problems during downstream processing [40].

Although Tanfloc has been demonstrated to flocculate marine microalgae [29], low flocculation was observed in this study as a consequence of the high pH (~10) of the medium. Likewise, Selesu *et al*. [41] achieved a flocculation efficiency of only 30% using Tanfloc for harvesting microalgae *Scenedesmus* sp. at pH 11. Having a point of zero charge of 8.17, Tanfloc assumes a neutral surface charge at higher pH and, consequently, loses its ability to flocculate either through charge neutralization or bridging [29]. Similarly, the conditions of the culture medium did not favor biomass flocculation using chitosan. At pH > 8, the amine groups on the surface of chitosan get deprotonated, which makes it impossible for chitosan to neutralize the microalgal surface charges to induce flocculation by charge neutralization or bridging. Moreover, the high ionic strength of the medium would result in coiling of the polymer [42,43]. Blockx *et al.* [20] reported that chitosan can also induce flocculation of microalgae at high pH (>7.5) and in seawater medium, but in that case flocculation occurs via sweeping mechanism and much higher doses of chitosan are needed than in freshwater

conditions (>75 mg L^{-1}). Similarly, Farid *et al.* [21] reported higher flocculation efficiencies of chitosan at high pH (9) when compared to neutral pH (7) with marine microalgae *Nannochloropsis* sp. However, no sweeping mechanism was observed in this study with chitosan doses up to 200 mg g^{-1}.

Another important parameter in flocculation is the biomass concentration factor. Less concentrated biomass flocs will require a secondary dewatering process. Maximizing the quantity of culture medium that can be recycled and managing lower volumes of biomass is essential in terms of process economics [44]. Flocculation with Zetag resulted in a maximum biomass concentration factor of 6.5 at a dose of 40 mg g^{-1}, while flocculation with MIM-*g*-CNCs exhibited a concentration factor of only 3.8 at a similar dose (Supplementary material, Fig. S2). Biomass concentration factors in the range of 3.5 – 14.1 have been reported for different cationic polymers while harvesting marine microalgae by flocculation followed by 2 hours gravity settling [17]. However, concentration factors obtained in this study were less than those reported by Eyley *et al*. [24] who achieved concentration factor as high as 49 with freshwater microalgae *C. vulgaris*, harvesting by cationic CNCs-based flocculation and 30 min of gravity settling.

3.2. Flocculation during semi-continuous cultivation and repeated recycling of spent medium

In a photosynthetic biogas upgrading process, the spent medium after biomass harvesting is recycled to the photobioreactor through an absorption column to remove the CO_2 and H_2S from the biogas. In this context, it is important to evaluate the impact of flocculation on biomass growth after recycling. Based on the previous results of this study, Zetag and MIM-*g*-CNCs were selected to study their effect during repeated recycling of spent medium. The impact of these flocculants on biomass growth was compared with that of centrifugation.

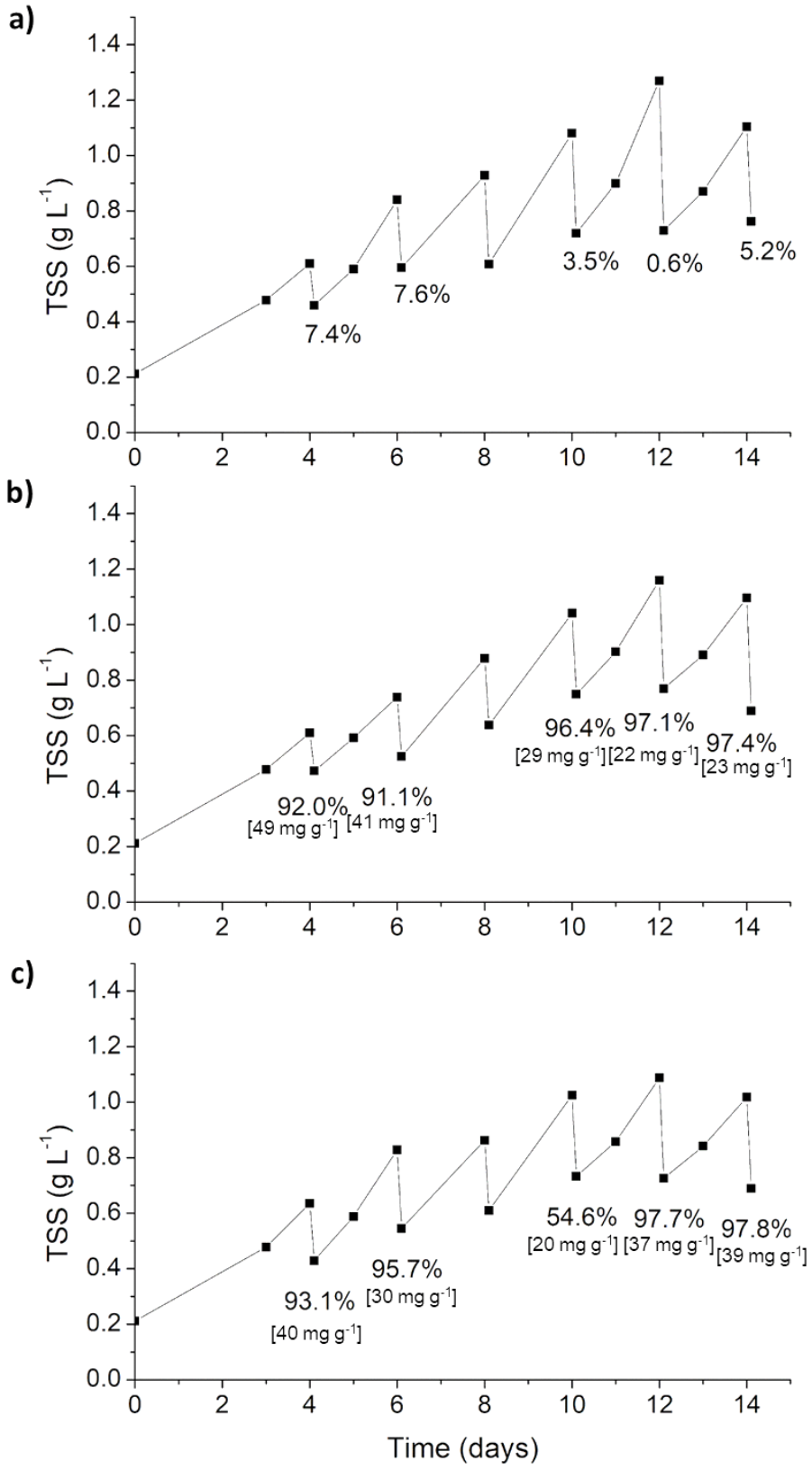

Fig. 2. Growth curve of the microalgal-bacterial consortium in the recycling medium with a) centrifugation (control) and flocculation with b) Zetag and c) cationic cellulose nanocrystals. The values below represent the flocculation efficiencies (%) and dose of flocculants (mg g^{-1}) during each harvesting cycle.

Spontaneous settling of microalgal-bacterial biomass (after 30 min) without flocculants was negligible, ranging between 1 – 8% over all harvesting cycles tested. Addition of Zetag and MIM-*g*-CNCs resulted in maximum flocculation efficiencies of ~97% at a dose of 23 mg g^{-1} and ~98% at 39 mg g^{-1}, respectively. Different flocculant doses were tested in the subsequent harvesting cycles in order to determine the minimum dose of flocculant. Flocculation with Zetag resulted in a flocculation efficiency of 97% with doses as low as 22 mg g^{-1}, whereas, with MIM-*g*-CNCs, a dose of 20 mg g^{-1} only achieved 55% of flocculation (Fig. 2).

A steady growth of microalgal-bacterial biomass was observed during semi-continuous cultivation using all three harvesting methods (centrifugation, Zetag, and MIM-*g*-CNCs-based flocculation), over 5 cycles of repeated recycling of 500 mL culture medium. Harvesting by centrifugation resulted in a 5 – 9% increased biomass growth when compared to flocculation-based harvesting (Fig. 2). Specific growth rates differed between the different harvesting treatments and along the time course of cultivation (Fig S3, supplementary material). Zetag being a synthetic polyacrylamide polymer and MIM-*g*-CNCs possessing an aromatically dislocated positive charge could be toxic to microalgae at high concentrations. In this regard, although slightly lower growth rates were observed in the last harvesting cycles using Zetag and MIM-*g*-CNCs in comparison with harvesting based on centrifugation, no detrimental effect on microalgae growth was observed along the 5 cycles. Moreover, concentrations of these flocculants were optimized to minimize the dose required to induce flocculation and to avoid the presence of free polymers in the recycled medium. This was verified through ζ-potential analysis of cell free supernatant before and after harvesting at each cycle (Supplementary material, Table S4). The presence of free flocculant in the spent medium should be evident from an increase in ζ-potential in the spent medium. In this study, no significant change in the ζ-potential of the spent medium was observed between centrifugation, Zetag, and MIM-*g*-CNCs -based flocculation, demonstrating that the quantity of flocculant that was returned to the cultivation system was minimal (Supplementary material, Table S4). During the recycling experiments, an increase in the pH of the culture medium (from 10 to 10.8) and a decrease in the inorganic carbon concentration (from 1798±0 to 913±69 mg L^{-1}) were observed as a result of the photosynthetic activity of the microalgae without CO_2 addition (Table S4,

supplementary material). Flocculation did not affect the pH, which is essential for effective biogas upgrading using microalgae.

Moreover, flocculation was uniform and was not selective to particular microalgal species of the consortium. As observed by microscopic analysis, no change in the microalgae community was found during any of the recycling experiments. *Chlorella* sp. and *Oscillatoria* sp. continuously dominated the consortium along with uncharacterized bacterial species.

3.3. Biomass separation after flocculation

Following flocculation, separation of biomass flocs from the culture medium is an important process step. The biomass concentration factor is an indicator of the efficiency of biomass separation. Separation was achieved by gravity sedimentation of the flocs for 30 min. The biomass concentration factor during repeated recycling experiments was lower than the ones observed during dose-response experiments (refer to section 3.2.). Zetag-based flocculation resulted in concentration factors in the range of 3.2 – 7.7, whereas MIM-*g*-CNCs-based flocculation resulted in a maximum concentration factor of only 2.0 (Fig. 3; supplementary material, Table S4). The higher concentration factors obtained for Zetag as the flocculant in comparison to MIM-*g*-CNCs could be attributed to a larger floc size and more compact structure as generated with the former (Fig. 3). In this context, Zhang *et al*. [45] proposed that not only the size of the flocs has influence on the settling velocity and the concentration factor of the microalgal biomass, but also the structure of these flocs, where microalgal flocs with large and compact structure should settle better under gravity.

In order to improve the concentration factor, screening was evaluated as a separation method. The biomass flocs obtained with Zetag and MIM-*g*-CNCs were allowed to settle for 30 min and screened through a nylon mesh screen with a pore size of 180 µm. Microalgal-bacterial culture without flocculants (acting as a control) resulted in harvesting efficiencies of 18% and 24% following 30 min settling and 180 µm screening, respectively. The cell size of microalgae in this consortium varied between 0.5-200 µm. Without flocculation, most of the cells crossed the 180 µm screen. In addition, a 30 µm pore size screen was also tested, but this was not efficient due to clogging of the mesh. On the other hand, Zetag-based flocculation resulted in harvesting efficiencies of 97% for both, settling and 180 µm screening. Similarly, MIM-*g*-CNCs-based flocculation resulted in harvesting efficiencies of 98% and 95% for settling and

180 µm screening, respectively (Fig. 4). The slight lower harvesting efficiency for MIM-*g*-CNCs with a 180 µm screen could be due to the fact that some smaller flocs or individual cells that were not flocculated passed through the screen. In this context, Verfaillie *et al*. [26] reported a low harvesting efficiency when using flocculation with cationically-modified CNCs followed by screening through a mesh with pore size of 180 µm due to unstable structural integrity of the flocs.

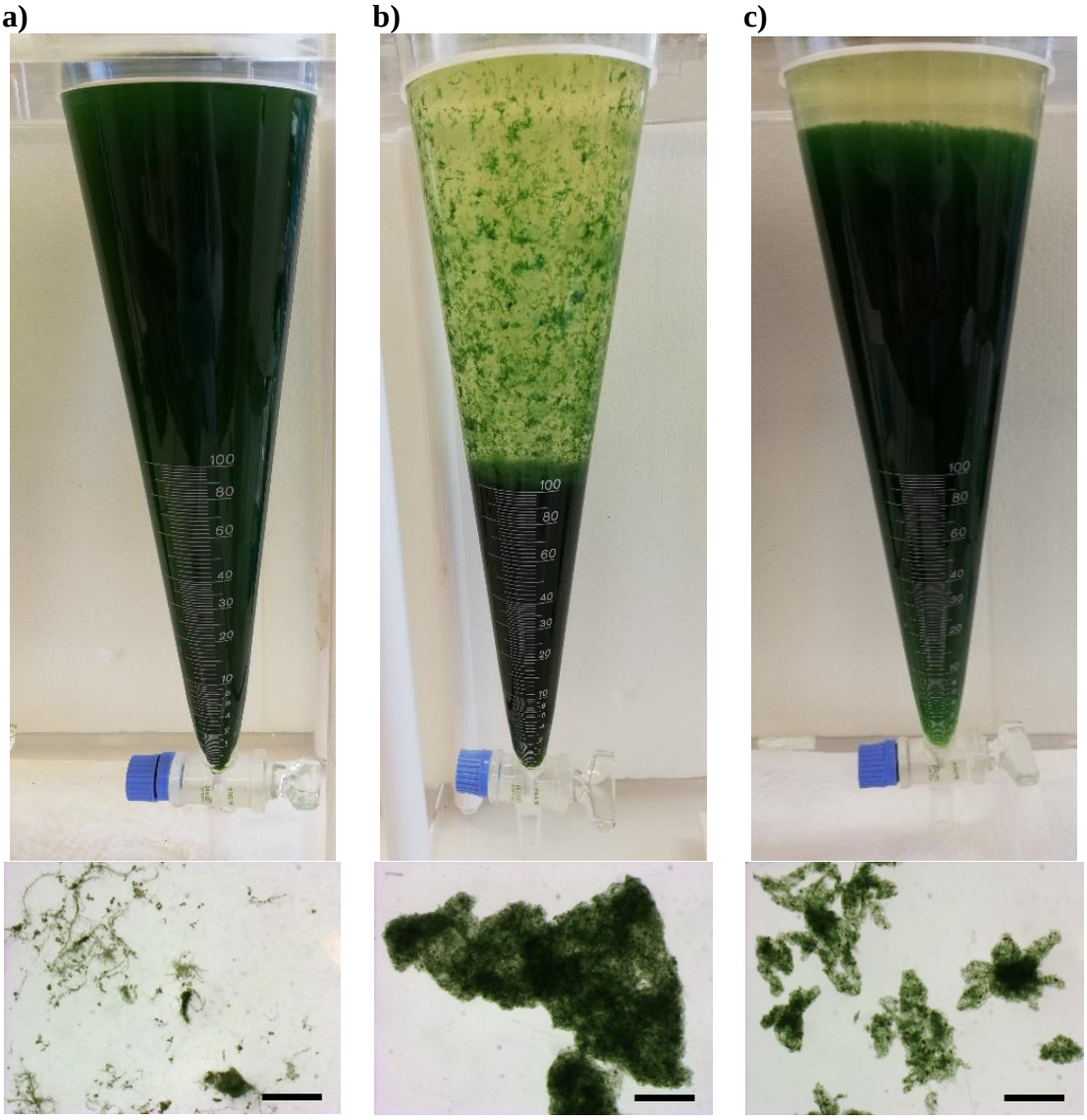

Fig. 3. Concentration of biomass flocs in Imhoff cone after 30 min settling during the repeated recycling experiments and microphotographs of flocs formed during a) gravity settling for 30 min, b) Zetag-based flocculation and c) cationic cellulose nanocrystals-based flocculation. Scale bar represents 250 µm.

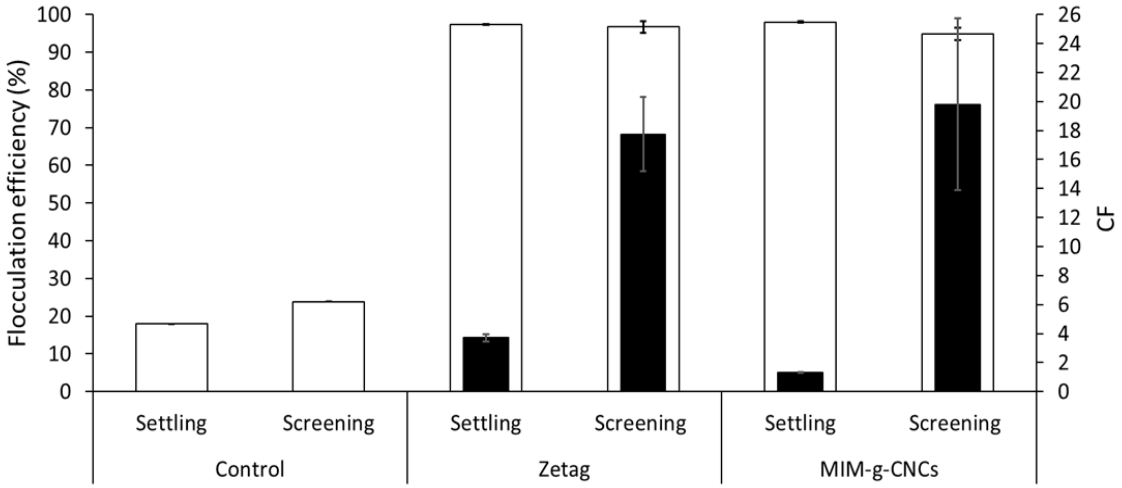

Fig. 4. Comparison of the harvesting efficiency (white bars) and concentration factor (CF; black bar) (average and standard deviation; n=2) of control (without flocculant), Zetag and cationic cellulose nanocrystals (MIM-*g*-CNCs)-based flocculation under different solid-liquid separation methods (gravity settling and screening with nylon mesh screen of pore size 180µm).

Screening resulted in higher biomass concentration factors (up to 19.8; Fig. 4) compared to those for centrifugation (maximum value of 10; supplementary material, Table S4). With Zetag-based flocculation, concentration factors of 3.7 and 17.7 were obtained for 30 min settling and 180 µm screening, respectively. With MIM-*g*-CNCs-based flocculation, a concentration factor of 19.8 was obtained with screening. This value is ~15 times higher than the concentration factors obtained with gravity settling (1.3; Fig. 4). Hwang *et al*. [46] reported a maximum concentration factor of 25 using a cross-flow membrane filtration system of polyethylene terephthalate with a pore size of 4 µm using a 3% of polyvinyl alcohol as coating material for harvesting *Chlorella* sp. Monte *et al*. [47] obtained a concentration factor of 4.8 with a loss of integrity of 10% while harvesting *Dunaliella salina* using a microfiltration membrane with a nominal pore size of 0.1 µm made of polyethersulfone.

In spite of demanding slightly higher energy costs (0.4 kWh/m^3 for screening *vs* 0.1 kWh/m^3 for gravity settling) [48], considering the advantages of achieving a high biomass concentration in a short time, screening using a 180 µm nylon mesh could be a good alternative to gravity sedimentation after flocculation.

4. Conclusions

In this study, five different flocculants were tested to harvest microalgal-bacterial biomass from a photosynthetic biogas upgrading process. Zetag and MIM-*g*-CNCs resulted in flocculation efficiencies >92% at 30 and 20 mg g^{-1}, respectively. Both

flocculants were effective in harvesting biomass under semi-continuous cultivation with repeated recycling of spent medium. Moreover, both Zetag and MIM-*g*-CNCs did not result in any detrimental effect on either microalgal growth or pH of the spent medium during 5 cycles of harvesting. Finally, screening of the biomass flocs with a nylon mesh with 180 µm pore size was demonstrated to achieve high biomass concentration factors. This flocculation-based harvesting is rapid and efficient in solid-liquid separation and hence could be applied in current biogas upgrading processes to replace the traditional gravity settlers-based harvesting.

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Supplementary Material

Harvesting microalgal-bacterial biomass from biogas upgrading process and evaluating the impact of flocculants on their growth during repeated recycling of the spent medium

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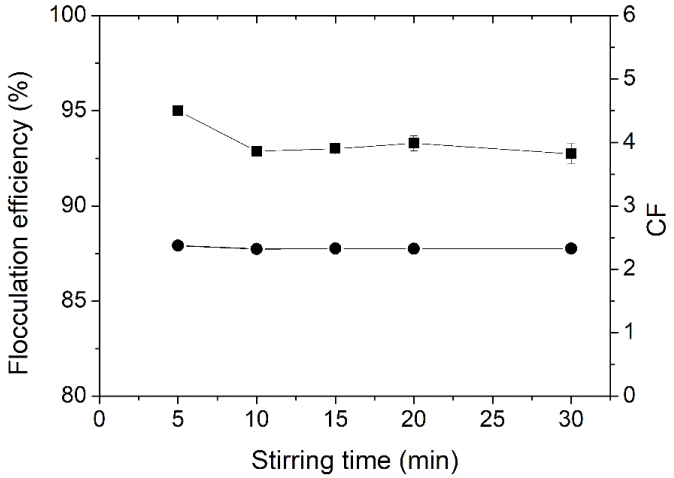
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15
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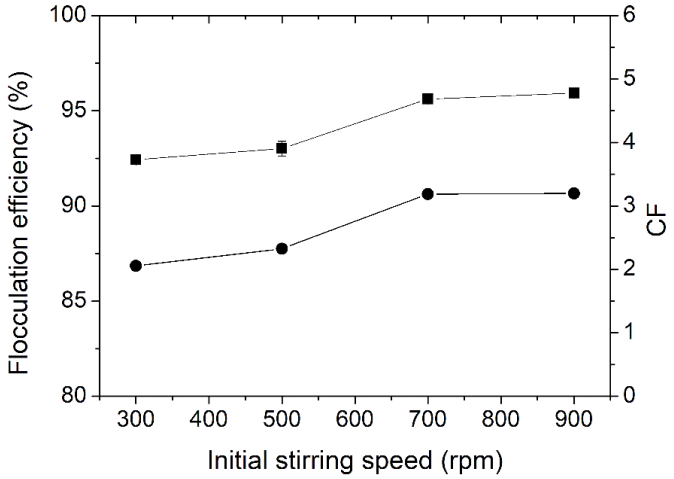
25
30

b)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800
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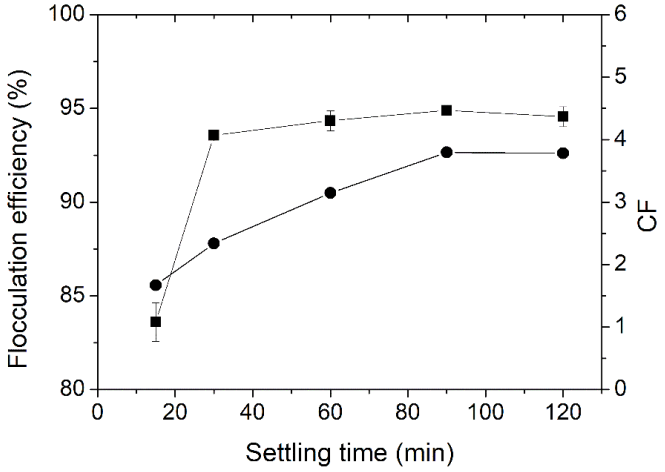
c)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2
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0
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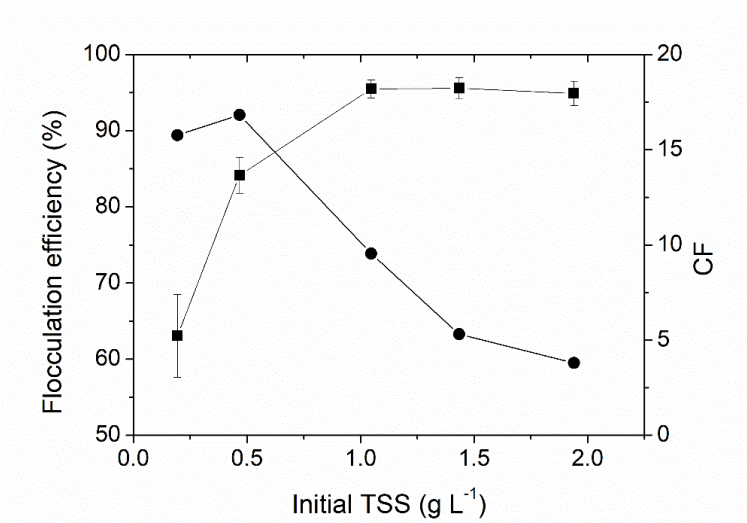
60
80
100
120

d)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10
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0.0
0.5
1.0
1.5
2.0

e)

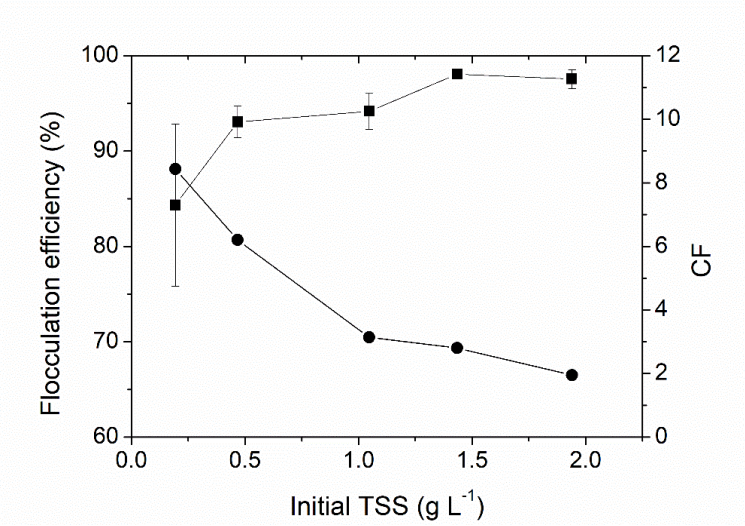

Fig. S1. Influence of the different flocculation conditions: a) stirring time, b) initial stirring velocity, c) settling time, d) biomass concentration (Zetag) and e) biomass concentration (cationic cellulose nanocrystals) on the flocculation efficiency (■) and concentration factor, CF (●) (average values and their standard deviation; n=2).

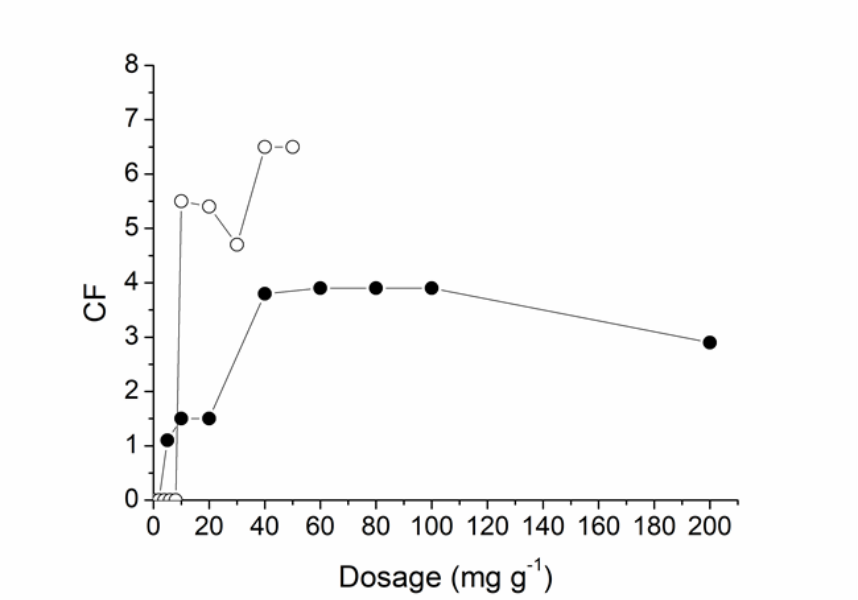

Fig. S2 Concentration factor (CF) of dose-response curves of Zetag (○) and cationic cellulose nanocrystals (●).

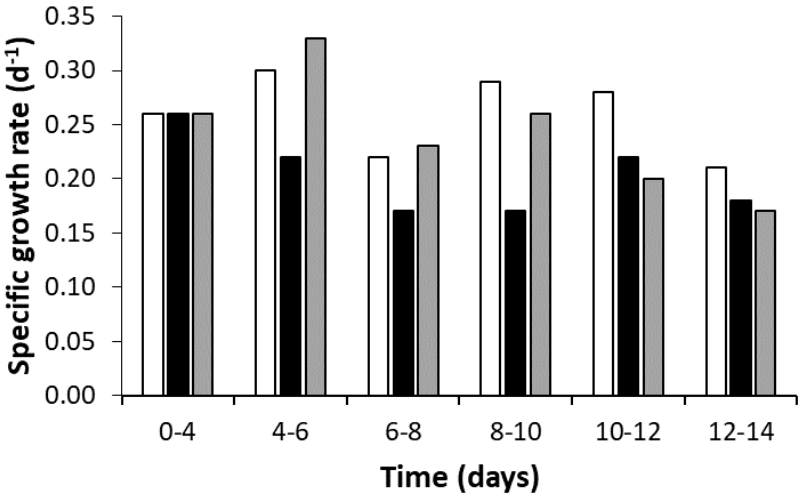

Fig. S3 Specific growth rate of the microalgae consortium during the repeated recycling experiments with centrifugation (white) and flocculation with Zetag (black) and cationic cellulose nanocrystals (grey).

Table S4 Parameters monitored during the repeated recycling experiments (pH, ζ-potential (average values and their standard deviation; n=3), inorganic carbon concentration (IC), concentration factor (CF) after 30 min of settling and volume of mineral medium added after harvesting).

	Recycling	**Day**	**pH**		**ζ-potential**		**IC**	**CF**	**V,**
			Before	**After**	**Before**	**After**	**mg L^{-1}**		**ml**
Centrifugation	1st	4	10.00	10.06	-28.14±1.33	-31.09±0.79	1798	8.3	60
	2nd	6	10.20	10.12	-38.02±2.41	-31.85±1.60	1712	10	50
	3rd	10	10.80	10.42	-23.63±0.17	-31.1±0.19	1455	10	50
	4th	12	10.30	10.15	-27.17±1.84	-37.05±1.55	1070	7.1	70
	5th	14	10.25	10.20	-21.86±0.93	-30.31±3.58	835	10	50
Cationic cellulose nanocrystals (MIM-*g*-CNCs)	1st	4	10.04	10.06	-28.13±1.33	-28.15±1.66	1798	1.4	180
	2nd	6	10.24	10.12	-25.22±4.59	-28.03±2.82	1712	1.4	210
	3rd	10	10.77	10.40	-28.56±2.13	-32.07±1.99	1627	0.6	50
	4th	12	10.26	10.12	-27.17±1.84	-28.45±1.10	1027	1.5	180
	5th	14	10.12	10.10	-21.86±0.93	-31.26±1.17	963	2.0	160
Zetag	1st	4	10.04	10.02	-28.13±1.33	-25.90±1.18	1798	7.7	60
	2nd	6	10.24	10.14	-24.85±1.20	-29.93±1.19	1669	3.3	140
	3rd	10	10.77	10.34	-33.98±2.64	-24.77±1.62	1498	3.2	150
	4th	12	10.27	10.14	-29.42±1.80	-32.05±0.78	1070	3.2	150
	5th	14	10.21	10.17	-29.35±2.42	-24.23±0.63	942	5.7	85

Chapter 9

Conclusions and future work

The optimization of photosynthetic biogas upgrading to achieve a biomethane complying with national and international standards coupled to wastewater treatment in a HRAP interconnected to a biogas absorption column was successfully carried out in this thesis at pilot and semi-industrial scale.

The influence of the alkalinity and temperature of the cultivation broth was systematically evaluated in **Chapter 3** in order to improve the efficiency of the process. Alkalinity was here identified as a key environmental parameter exerting an impact on CO_2 removal from biogas. In this context, biomethane composition complied with most international standards for biogas injection into natural gas grids or use as a vehicle fuel only when photosynthetic biogas upgrading was carried out at high alkalinity (inorganic carbon concentrations of ~1500 mg C L^{-1}). Otherwise, low alkalinity media (~100 mg inorganic carbon L^{-1}) entailed a low CO_2 mass transfer from biogas due to the rapid acidification of the scrubbing liquid in the absorption column, which might induce inorganic carbon limitations in the culture broth. On the other hand, a negligible effect of the temperature in the range of 12-35ºC on the quality of the upgraded biogas was recorded at high-medium alkalinity, while low temperatures favoured CO_2 removal at low alkalinity.

Since alkalinity in the cultivation medium played a key role on the efficiency of CO_2 removal in the biogas absorption column, the long-term impact of high alkalinity on CO_2 fixation by microalgae was evaluated in **Chapter 4**. Although biogas upgrading was more effective and robust at inorganic carbon concentrations in the cultivation broth higher than 2400 mg C L^{-1}, this high salt content negatively impacted on the photosynthetic activity of microalgae as a result of oxidative stress. Furthermore, higher alkalinities entailed a higher CO_2 stripping, thus lowering the environmental advantage of this biotechnology. Finally, the influence of biomass concentration (0.33-1.38 g SSV L^{-1}) on biomethane quality and microalgae growth was also assessed. High biomass concentrations mediated a slight decrease on the CO_2 gas-liquid mass transfer in the absorption column and decreased biomass productivities in the HRAP.

Chapter 5 was focused on the semi-industrial validation of the simultaneous photosynthetic biogas upgrading and wastewater treatment under outdoor conditions. The effectiveness of photosynthetic biogas upgrading was low when using domestic wastewater as a nutrient source regardless of the hydraulic retention time in the HRAP (3.5 and 8 days), while the use of centrate enhanced CO_2 and H_2S removals due to its

higher pH and alkalinity. The influence of biogas flowrate from 274 to 459 L h^{-1} at similar liquid to biogas (L/G) ratio in the absorption column was negligible. Otherwise, higher L/G ratios supported higher CO_2 and H_2S removals along with higher N_2 and O_2 stripping from the cultivation broth to the biogas upgraded, which resulted in a lower biomethane quality. Finally, an efficient nutrient removal in the wastewaters was reached regardless of the operational conditions.

In **Chapter 6**, an innovative control strategy based on the regulation of the recycling liquid flowrate, and indirectly the L/G ratio, to meet the target biomethane quality during photosynthetic biogas upgrading was successfully developed. The control system implemented was able to assure a CO_2 and O_2 content lower than 2.5% and 1%, respectively, and negligible concentrations of H_2S under biogas flowrate fluctuations ranging from 60 to 120 ml min^{-1} regardless of the temperature and the alkalinity of the cultivation broth at pH 10. On the contrary, the low CO_2 removal recorded at pH 8.5 together with the increase in O_2 concentrations in the upgraded biogas due to the high L/G ratios imposed by the control system, entailed opposite control responses. This confirmed that pH was a critical operating parameter in this technology. The control strategy was further evaluated at semi-industrial scale in **Chapter 7**. In this work the control system was able to maintain CO_2 concentrations <2% and O_2 concentrations <1% in the biomethane regardless of the pH (9.05-9.50) and fluctuations in the biogas flowrate between 143 and 420 L h^{-1}. Although this green biotechnology typically exhibits a poor robustness against failures in biogas and liquid supply, the control system provided a suitable biomethane quality after a shutdown and resumption of biogas supply or liquid recirculation in the absorption column. This control strategy validated at pilot and semi-industrial scale can provide a satisfactory biomethane quality and overcome the negative impact of operational failures or environmental variations on photosynthetic biogas upgrading performance.

A final investigation was carried out to achieve an efficient separation of the microalgal-bacterial biomass produced during photosynthetic biogas upgrading via flocculation (**Chapter 8**). In this context, only Zetag 8125 and cationically modified cellulose nanocrystals (CNCs) resulted in flocculation efficiencies >90% among the five flocculants tested. Moreover, these flocculants did not have a pernicious impact on the algal culture when the biomass-free cultivation broth was recycled. Moreover, screening with a nylon mesh of 180 μm pore size after flocculation was more efficient and less

time-consuming than gravity settling. This flocculation-based harvesting is a promising alternative to conventional gravity settling in photosynthetic biogas upgrading processes.

Based on the outcomes and limitations found in this thesis, further research on valorisation alternatives should focus on:

- The enrichment of high performance microalgae and bacteria consortia able to grow and effectively sequester CO_2 from biogas and nutrients from digestates under the extreme conditions of alkalinity and pH needed during photosynthetic biogas upgrading.
- The development of cost-effective strategies to reduce the desorption of N_2 and O_2 from the cultivation broth prior to the absorption column, which could allow operating at higher L/G ratios under unfavorable CO_2 absorption conditions (i.e. low pH or alkalinity) without an undesirable increase in the O_2 and N_2 content in the biomethane.
- Optimization of photobioreactor configuration in order to enhance CO_2 capture by the microalgae at low operational and investment costs.
- Research on manufacture of value-added products from the microalgal-bacterial biomass obtained as by-product in this process to further enhance its economic viability.
- Continuous implementation of flocculation followed by a separation step as harvesting method during photosynthetic biogas upgrading.
- One-year continuous evaluation of the full-optimized system at semi-industrial scale.

Chapter 10

About the author

Bibliography

María del Rosario Rodero Raya (Jaén, 1990) started a Chemical Engineering degree in 2008 at the University of Granada. In November 2013, she obtained a 8-months scholarship from the Spanish Ministry of Education, Culture and Sports as collaborating researcher at the Department of Analytic Chemistry of the University of Granada. Then, she carried out (January-June 2015) a 6-months internship at the company Marquesado Solar S.L., owner of the Solar Thermal Power Plant Andasol 3 (Guadix, Spain).

In October 2016, Mª del Rosario joined the VOC & Microalgae Research Group headed by Associate Professor Raúl Muñoz in the Environmental Technology Research Group (Institute of Sustainable Processes – University of Valladolid). Mª del Rosario carried out her PhD research within the INCOVER project funded by European Union's Horizon 2020 research and innovation programme (689242). Her PhD studies focused on the optimization of an innovative biogas upgrading system in open algal-bacterial photobioreactors and its evaluation at large scale. The candidate carried out within her PhD studies 3 research stays (July 2017– February 2018, September 2018-December 2018, April 2019-September 2019) at Wastewater Treatment Plant "El Torno" belonging to the multinational company FCC Aqualia, where she operated a semi-industrial high rate algal pond interconnected to a biogas absorption column. Finally, she also carried out a 3-months research stay (January 2019 – March 2019) at the Department of Biology of KU Leuven, Campus Kulak (Kortrijk, Belgium) with the purpose of achieving an effective biomass harvesting during photosynthetic biogas upgrading under the supervision of Dr. Koenraad Muylaert and Dr. Ramasamy Praveenkumar.

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1. Rodero, M. del R., Posadas, E., Toledo-Cervantes, A., Lebrero, R., Muñoz, R., 2018. Influence of alkalinity and temperature on photosynthetic biogas upgrading efficiency in high rate algal ponds. Algal Res. 33, 284–290. doi:10.1016/j.algal.2018.06.001.
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2. Ángeles, R., Rodero, M. del R., Carvajal, A., Lebrero, R., Muñoz, R., 2019. Potential of Microalgae for Wastewater Treatment and Its Valorization into Added Value Products. In: Application of Microalgae in Wastewater Treatment, pp. 281-315. doi:10.1007/978-3-030-13909-4_13

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1. Posadas, E., Rodero, M. del R., Marin, D., Lebrero, R., García-Encina, P.A., Muñoz, R. Revisiting Microalgae-based Processes for a Cost-effective and Integral Wastewater Treatment. Workshop Leading Edge Technologies for the Removal of Emerging Pollutants. 24 November 2016, Madrid, Spain. (**Oral Presentation**).
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10. Rodero, M. del R., Lebrero, R., Serrano, E., Lara, E., Arbib, Z., Muñoz, R. Biogas upgrading using algal-bacterial processes in wastewater treatment plants. 16th IWA World Conference on Anaerobic Digestion. 23-27 June 2019, Delft, The Netherlands. (**Poster + oral presentation**).

11. Rodero, M. del R., Carvajal, A., Castro, V., Navia, D., de Prada, C., Lebrero, R., Muñoz, R. Development of a control strategy to cope with biogas flowrate variations during photosynthetic biogas upgrading. IWA Conference on Algal

Technologies and Stabilization Ponds for Wastewater Treatment and Resource Recovery. 1-2 July 2019, Valladolid, Spain. (**Oral presentation**).

Long-term research stays

- WWTP "El Torno", FCC Servicios Ciudadanos (AQUALIA), Chiclana de la Frontera (Spain). July 2017 - February 2018; September 2018 - December 2018; April 2019 - September 2019.
- Laboratory for Aquatic Biology, KU Leuven, Campus Kulak, Kortrijk (Belgium). January 2019 - March 2019. Supervisors: Professor Koenraad Muylaert and Dr. Ramasamy Praveenkumar.

Participation in Research Projects

- Innovative Eco-Technologies for Resource Recovery from Wastewater (INCOVER). European Union's Horizon 2020 research and innovation programme (689242). P.I: Raúl Muñoz and Raquel Lebrero.

Fellowships

1. UVa-Predoctoral researcher Fellowship (2016).
2. Travel and accommodation grant for the participation in the 2nd EUALGAE Training School: "Microalgae processes: from fundamentals to industrial scale" (2017). COST Action ES1408 "European network for algal-bioproducts (EUALGAE)".
3. Travel and accommodation grant for the participation in the 2nd EUALGAE workshop of algae bioproducts for early career investigators (2018). EUALGAE COST Action ES1408.
4. International Mentor Program 2018-19 of IMFAHE Foundation.
5. Short Term Scientific Mission Grant for research stay in KU Leuven-Campus Kulak, Kortrijk (2019). EUALGAE COST Action (ES1408-41358).
6. Travel, accommodation and registration grant for taking an English course in the European School of English Malta (2019). ERASMUS + programme (KA103).

Teaching

- Environmental and Process Technology. Assistant Professor, 2 ECTS. Chemical Engineering Degree. 1st course, academic year 2017/2018, University of Valladolid (Spain).

Conference committee

- Member of the Organizing Committee of: "IWA Conference on Algal Technologies and Stabilization Ponds for Wastewater Treatment and Resource Recovery". 1-2 July 2019, Valladolid, Spain.

Peer-review in ISI Web of Knowledge journals

- Journal of Environmental Management
- Journal of Water Environment Research

Attended short-courses and seminars

1. Complementary subject "Technology for water treatment". Environmental Engineering Master. University of Valladolid. 10/2016 – 01/2017. 6 ECTS.
2. Complementary subject "Prevention and treatment of atmospheric pollution". Environmental Engineering Master. University of Valladolid. 10/2016 – 01/2017. 5 ECTS.
3. Course "Redacción de Artículos Científicos en Ingeniería". University of Valladolid. 16/01/2017. 4 hours.
4. English Course. Level B2. University of Valladolid. 30/01/2017 – 05/04/2017. 50 hours.
5. Course "Coaching. El arte de ser professional". University of Valladolid. 29/02/2017 and 07/03/2017. 8 hours.
6. Course "Introducción a la edición de textos con LATEX Composición y presentaciones con Beamer". University of Valladolid. 02/05/2017 –24/05/2017. 30 hours.
7. Course "Biotecnología de microalgas". University of Valladolid. 08/05/2017 – 12/05/2017. 10 hours.
8. English Course. Level B2. European School of English Malta. 16/09/2019 – 20/09/2019. 22.5 hours.
9. Course "Taller Práctico sobre Técnicas Analíticas Físico-Químicas e Instrumentales". University of Valladolid. 06/11/2019 –16/11/2019. 8 hours.

10. Course “Iniciación al análisis de secuencias 16 S Illumina® MiSeq para el estudio de comunidades bacterianas”. University of Valladolid. 16/12/2019 – 19/12/2019. 14 hours.
11. Course “Eficiencia y viabilidad de nuevas tecnologías en procesos de tratamiento de aguas residuales”. University of Valladolid. 09/03/2020 – 12/03/2020. 10 hours.
12. Course “Formación en Comunicación y Soft Skills”. University of Valladolid. 12/11/2020 - 20/11/2020. 8 hours.
13. Course “Introducción a las tecnologías de secuenciación y bases de datos genómicas”. University of Valladolid. 10/12/2020 –11/12/2020. 5 hours.

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