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Optimization of photosynthetic biogas upgrading combined with algal biomass valorization: assessing alternative operating strategies and photobioreactor configurations

Presentada por **Roxana Ángeles Torres** para

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Como Directores de la Tesis Doctoral titulada *"Optimization of photosynthetic biogas upgrading combined with algal biomass valorization: assessing alternative operating strategies and photobioreactor configurations"* realizada por Dª Roxana Ángeles Torres alumna del Programa de Doctorado Ingeniería Química y Ambiental, **autoriza** su presentación, considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresan su conformidad con dicha presentación.

D./Dª Raquel Lebrero Fernández y D. Raúl Muñoz Torre (*Directores de la tesis doctoral*):
X Declaran que cumplen los requisitos para poder ejercer la dirección de la tesis doctoral que establecen el RD 99/2011 (modificado por el RD 195/2016) y el Acuerdo del Comité de Dirección de la Escuela de Doctorado de la Universidad de Valladolid del 17 de febrero de 2014, es decir, que posee "al menos un período de actividad investigadora reconocido de acuerdo con las previsiones del RD 1086/1989 de 28 de agosto"

Valladolid, …… de…………………………… de 2021

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Resumen

Los combustibles fósiles son una fuente de energía no renovable que representa actualmente un 80 % de la demanda total de energía global, además de suponer una de las mayores contribuciones a las emisiones de gases de efecto invernadero (GEI). Con el objetivo de reducir el consumo de combustibles fósiles y las emisiones de GEI asociadas, numerosas legislaciones a nivel mundial se han enfocado en promover la producción de energías renovables y la lucha contra el cambio climático. A este respecto, la generación de energía renovable a escala global ha ido aumentando en las últimas décadas, alcanzando 2,378 Gigavatios de capacidad de potencia instalada a nivel mundial en 2018, lo que indica que la transición de energías fósiles a renovables está en auge. El biogás constituye uno de los subproductos de la digestión anaerobia de residuos con mayor potencial de revalorización como energía renovable por su alto contenido de metano (CH_4, 40 – 70 % v/v). Además, se trata de una materia prima adecuada para la producción de un biocombustible de calidad similar al gas natural, denominado biometano. Sin embargo, previo a su utilización, el biogás debe ser purificado (una etapa conocida como *upgrading*) debido a la presencia de gases contaminantes como dióxido de carbono (CO_2, 15 – 60 % v/v), sulfuro de hidrógeno (H_2S, 0.005 – 3 % v/v), nitrógeno (N_2, 0 – 2 % v/v), oxígeno (O_2, 0-1 % v/v), siloxanos (0 – 0.02 % v/v), compuestos orgánicos volátiles (COVs, < 0.6 % v/v), amoníaco (NH_3, < 1 % v/v) o monóxido de carbono (CO, < 0.6 % v/v). En la actualidad, las tecnologías comerciales disponibles a escala industrial para convertir el biogás a biometano se basan en procesos físico-químicos, que conllevan un alto consumo de reactivos químicos y energía, y elevados costes de operación. Algunas de las más empleadas son los lavadores de agua a presión, lavadores químicos, lavadores con solventes orgánicos, sistemas de adsorción PSA, membranas o separadores de CO_2 criogénicos.

Para superar las limitaciones de las tecnologías de *upgrading* físico-químico, en las últimas décadas se han desarrollado tecnologías biológicas capaces de eliminar uno o varios de los contaminantes presentes en el biogás con menores costes de operación e impactos medioambientales. Una de las alternativas más prometedoras, sostenible y respetuosa con el medio ambiente está basada en el uso de fotobiorreactores (FBRs) de microalgas y bacterias para la eliminación

simultánea de CO_2 y H_2S. En este proceso, los microorganismos fotosintéticos utilizan la luz solar incidente en el FBR para la fijación en forma de biomasa del CO_2 transferido desde el biogás, con la consiguiente generación de O_2. Este O_2 generado *in-situ* en el FBR es utilizado por las bacterias oxidadoras de H_2S para su oxidación a SO_4^{2-}. El potencial de esta innovadora biotecnología se ha demostrado en FBRs abiertos convencionales de crecimiento de biomasa en suspensión (high rate algal ponds (HRAPs)) en condiciones de interior y exterior, tanto a escala de laboratorio como a escala piloto. Para promover la transferencia de los contaminantes desde el biogás al medio de cultivo, el FBR está interconectado a una columna de lavado de biogás, logrando eficiencias de eliminación >98 % para el CO_2 y una eliminación completa del H_2S. A pesar de los satisfactorios resultados obtenidos para la eliminación de ambos contaminantes, la composición del biometano resultante no cumple con la mayoría de los estándares internacionales para su inyección en redes de gas natural o su uso como combustible para automoción debido a la contaminación con N_2 y O_2. Estos gases son desorbidos desde el caldo de cultivo recirculante entre el FBR y la columna de lavado de biogás al biometano. En ese sentido se han reportado composiciones de biometano de CH_4 > 87 %, CO_2 0.9 – 9 %, N_2 0.7 – 9 %, and O_2 0 – 2 %.

A este respecto, en la presente tesis se investigaron dos nuevas estrategias de operación para minimizar el contenido de N_2 y O_2 en el biometano. La primera estrategia operacional consistió en la desgasificación del caldo de cultivo antes de alimentarlo a la columna de lavado de biogás interconectada al HRAP. Para ello se instaló una membrana de contacto líquido-gas de fibra hueca de polidimetilsiloxano (PDMS), la cual se operó a diferentes presiones de vacío. Se obtuvieron eficiencias de eliminación de oxígeno disuelto del caldo de cultivo ≥60 % operando a una presión de vacío entre 100 – 300 mbar, confirmándose la capacidad de la tecnología de membranas acoplada a la limpieza fotosintética del biogás para reducir el contenido de O_2 del biometano. La segunda estrategia operacional reveló una mejora en la eliminación de CO_2 y H_2S al aumentar la presión de operación en la columna de lavado de biogás. Sin embargo, fue necesaria una sobrepresión mínima de 2 atm para limitar la desorción de N_2 y O_2. Los resultados de ambas estrategias operativas alcanzaron un biometano de alta calidad (CH_4 > 87 %, CO_2 0.9 – 9 %, N_2 0.7 – 9 %, y O_2 0 – 2 %).

Por otro lado, si bien el potencial de purificación de biogás a través de la simbiosis algas-bacterias ha sido ampliamente validado y optimizado en configuraciones abiertas como los HRAPs, son pocos los estudios que se han centrado en la implementación de estos procesos en FBRs cerrados. Esta configuración de FBRs ofrece mayores eficiencias fotosintéticas, productividades de biomasa más altas, una menor contaminación del biometano con N_2. Además, la valorización posterior de la biomasa algal generada en los FBRs cerrados tubulares (menos expuestos a contaminación biológica que sus homólogos HRAPs) permite aumentar la sostenibilidad económica de esta tecnología. En este contexto, la segunda parte de la investigación de esta tesis se centró en evaluar el rendimiento de la purificación fotosintética de biogás junto con la producción de biomasa algal en un FBR tubular interconectado a una columna externa de lavado de biogás. Durante 395 días se evaluó y optimizó la influencia de la alcalinidad y de la limitación de nitrógeno (N) en el caldo de cultivo en la calidad del biometano generado y en la composición de la biomasa obtenida. Una alta alcalinidad (~1700 mg L^{-1}) en el caldo de cultivo aseguró una composición de biometano que cumplía con las regulaciones internacionales para su inyección en redes de gas natural o su uso como combustible vehicular. Por otra parte, no se observó un efecto perjudicial sobre la calidad del biometano cuando se implementaron los ciclos de limitación de N. Además, la limitación de N promovió un aumento en la cantidad de carbohidratos almacenada en la biomasa algal, alcanzando valores hasta 2 veces superiores a los obtenidos en condiciones nutricionales normales. Estos resultados respaldan la posibilidad de utilizar la biomasa algal producida como materia prima para la producción de bioetanol.

Esta línea de investigación de la tesis se complementó con un estudio del rendimiento del FBR tubular durante el periodo oscuro, cuando se detiene la fotosíntesis durante la purificación de biogás. Para ello, se evaluó la influencia del régimen de suministro de biogás (continuo vs. alimentación durante el periodo de luz) sobre la calidad del biometano y la composición bioquímica de la biomasa algal en un FBR tubular. Los resultados confirmaron que la oxidación del H_2S durante el período de oscuridad era debida a la actividad de las bacterias sulfuro-oxidantes desnitrificantes, que utilizaban como aceptor de electrones el NO_3^- producido por la oxidación de NH_4^+ durante el período de luz. Esta eliminación eficaz del H_2S también durante el periodo de oscuridad evitó una posible inhibición

de las microalgas inducida por la acumulación de H_2S en el caldo de cultivo. Sin embargo, se registraron eliminaciones de CO_2 inferiores como resultado de la acidificación del caldo de cultivo (pH ≤ 9) cuando se suministró biogás de manera continua durante 24 h (periodo de luz y oscuridad), si bien el contenido de carbohidratos se incrementó un ~30 % operando en este régimen de alimentación.

Muchos de los organismos fotosintéticos empleados en la purificación de biogás (microalgas y cianobacterias) son capaces de acumular productos de valor añadido bajo determinadas condiciones de operación, lo que podría aumentar considerablemente la rentabilidad de esta biotecnología. Estudios previos han demostrado el potencial de la cianobacteria *Nostoc muscorum* para acumular glucógeno (GL) y poli–β–hidroxibutirato (PHB) empleando CO_2 como fuente de carbono. En este sentido, la última parte de la tesis se centró en el estudio y optimización del crecimiento y la acumulación de GL y PHB en *N. muscorum* empleando el CO_2 procedente del biogás o de gases de combustión. El resultado obtenido demostró la capacidad de esta cianobacteria para crecer y acumular compuestos de valor agregado bajo altas concentraciones de CO_2 en la fase gaseosa, similares a las encontradas típicamente en el biogás proveniente de la digestión anaerobia (~30 % v/v). La limitación de N en el caldo de cultivo no tuvo un efecto negativo significativo sobre la eficiencia de eliminación de CO_2 (> 93 %), mientras que la privación de nutrientes promovió la síntesis de GL. Así, en condiciones de limitación de N y P, *N. muscorum* acumuló ~54 % en peso seco de GL, casi 36 veces superior al valor registrado en presencia de nutrientes. Por el contrario, no se observó una acumulación significativa de PHB en las condiciones estudiadas, probablemente debido a la alta concentración de CO_2 en la fase gaseosa (30 % *v/v)*, y como consecuencia de una alta disponibilidad de carbono inorgánico en el medio cultivo. En conclusión, *N. muscorum* podría ser una fuente de bajo costo de carbohidratos para la producción de bioetanol o productos químicos de base biológica acoplada a la eliminación del CO_2 proveniente del biogás o de gases de combustión.

Abstract

Fossil fuels account for 80 % of the total energy consumption worldwide, significantly contributing to the greenhouse gas (GHG) emissions. Therefore, many recently developed international policies have focused on mitigating climate change and promoting renewable energy production, with the objective to reduce GHG emissions and fossil fuel consumption. Renewable energy production has steadily increased within the last decades at a global scale, reaching 2,378 Gigawatts of installed power capacity worldwide in 2018. Among renewable energy sources, biogas from the anaerobic degradation of organic waste constitutes a sustainable energy vector due to its high CH_4 content (~40 to 75 % v/v). However, a preliminary purification stage (known as upgrading) is recommended prior biogas use owing to the presence of other gas pollutants such as carbon dioxide (CO_2, 15 – 60 % v/v), hydrogen sulphide (H_2S, 0.005 – 3 % v/v), nitrogen (N_2, 0 – 2 % v/v), oxygen (O_2, 0-1 % v/v), siloxanes (0 – 0.02 % v/v), volatile organic compounds (VOC, < 0.6 % v/v), ammonia (NH_3, < 1 % v/v) or carbon monoxide (CO, < 0.6 % v/v). Moreover, the removal of most of these biogas pollutants is mandatory if the biogas is intended to be used either as a vehicle fuel or for injection into natural gas grids. Water scrubbing and membrane CO_2 separation are nowadays the market-leading technologies for biogas upgrading, together with other commercially available physical-chemical technologies such as chemical and organic solvent scrubbing, pressure swing adsorption, or cryogenic CO_2 separation. Nevertheless, the high energy and chemicals consumption, the elevated operating cost, and the complex sequences required for integral biogas upgrading, still limit the environmental and economic sustainability of these technologies.

In order to overcome these limitations, biological technologies capable of removing one or more pollutants present in raw biogas at lower operating costs and reduced environmental impacts have been recently developed. One of the most promising alternatives for the simultaneous removal of CO_2 and H_2S is photosynthetic biogas upgrading in algal-bacterial photobioreactors (PBRs). This biological technology is based on the fixation of CO_2 by microalgae via photosynthesis using solar light and the concomitant oxidation of H_2S by sulphur-oxidizing bacteria utilizing the oxygen photosynthetically produced. The potential of this innovative technology has been demonstrated both indoors and outdoors in

laboratory and demo high-rate algal ponds (HRAP) interconnected to an external biogas scrubbing column (SC), achieving removal efficiencies up to 98 % for CO_2 and an almost complete H_2S abatement. Despite the satisfactory results obtained for CO_2 and H_2S removal, previous experiments did not result in a biomethane composition complying with most biomethane standards due to the contamination of the upgraded biogas with N_2 and O_2 (stripped out from the cultivation broth in the biogas SC). In this regard, biomethane compositions of $CH_4 > 87$ %, CO_2 0.9 – 9 %, N_2 0.7 – 9 %, and O_2 0 – 2 % have been reported.

The present thesis assessed two new operational strategies devoted to minimizing the content of N_2 and O_2 in the upgraded biogas. The first approach aimed at reducing the content of both gases in the biomethane via degassing of the culture broth before being fed to the biogas SC interconnect to the HRAP. A hollow fiber polydimethylsiloxane (PDMS) gas-liquid contactor membrane was installed and operated at different vacuum pressures, supporting dissolved oxygen removals ≥ 60 % from the cultivation broth at 100 – 300 mbar of vacuum pressure. Thus, the potential of membrane technology to reduce O_2 content in the upgraded biogas was confirmed. The second approach revealed an enhanced removal of CO_2 and H_2S from the biogas when increasing the operating pressure in the biogas SC. However, a minimum overpressure of 2 atm was necessary to limit O_2 and N_2 desorption. These results demonstrated that both strategies were capable of providing a high-quality biomethane also in terms of O_2 and N_2 content ($CH_4 > 87$ %, CO_2 0.9 – 9 %, N_2 0.7 – 9 %, y O_2 0 – 2 %).

Even though the potential of algal-bacterial symbiosis for biogas purification has been consistently studied and validated in open photobioreactor configurations such as HRAPs, there is a lack of studies assessing the implementation of photosynthetic biogas upgrading in enclosed PBRs. This photobioreactor configuration offers higher photosynthetic efficiencies, enhanced biomass productivities, and limited N_2 contamination of the biomethane. Additionally, further valorization of the algal biomass generated in the enclosed tubular PBRs (less prone to biological contamination than their HRAP counterparts) allows to increase the economic sustainability of this technology. In this context, the second part of this thesis was focused on the evaluation of the biogas upgrading performance coupled with the production of tailored algal biomass in a tubular PBR interconnected to an external biogas SC. The influence of alkalinity and nitrogen

(N)-deprivation was assessed and optimized for 395 days of continuous operation. A high alkalinity concentration of ~1700 mg L^{-1} in the cultivation broth ensured a biomethane composition complying with international regulations for biomethane injection into natural gas grids or use as vehicle fuel. Moreover, the implementation of N feast-famine cycles did not result in a detrimental effect on the biomethane quality. N-deprivation promoted the continuous storage of intracellular carbohydrate content, achieving values up to 2 times higher than those obtained under regular nutritional conditions. These results support the potential of using algal biomass as a feedstock to produce bioethanol.

The use of enclosed PBRs was thus validated as an alternative to the open configuration in order to prevent excessive N_2 stripping to biomethane and increase the CO_2 fixation capacity during biogas upgrading. However, there is limited knowledge on the performance of tubular PBRs during the dark periods, when photosynthesis stops. For this purpose, the influence of biogas supply regime (continuous *vs* feeding during the light period) on the biomethane quality and biochemical biomass composition was evaluated in an enclosed tubular PBR interconnected to an external biogas SC. The results confirmed that the effective H_2S oxidation during the dark period takes place by denitrifying bacteria using the NO_3^- produced from NH_4^+ oxidation during the light period as electron acceptor. This prevented microalgae inhibition induced by H_2S build-up in the cultivation broth. However, lower CO_2 removals were recorded as a result of the acidification of the cultivation broth (pH ≤ 9) when raw biogas was supplied continuously for 24 h, albeit the carbohydrate content increased by ~30 % under continuous biogas supplementation.

Most photosynthetic organisms used for biogas purification (microalgae and cyanobacteria) are able to accumulate value added products under certain operating conditions, which could boost the cost-effectiveness of this biotechnology. Preliminary studies have demonstrated the potential of the cyanobacterium *Nostoc muscorum* to accumulate value added products such as glycogen (GL) and poly–β–hydroxybutyrate (PHB) using CO_2 as the carbon source. In this context, the last part of the thesis focused on assessing and optimizing the growth and simultaneous accumulation of GL and PHB in *N. muscorum* using CO_2 from either biogas or flue-gas. The result obtained demonstrated the capacity of *N. muscorum* to grow under high CO_2 concentrations in the gas phase, similar to those

typically found in raw biogas from anaerobic digestion (~30 % *v/v*). Moreover, N-deprivation in the cultivation broth had no significant negative effect on CO_2 removal efficiencies (> 93 %), while promoting GL synthesis. *N. muscorum* accumulated GL up to ~54 % dcw under N and P-deprivation, almost 36 times higher than the values recorded under nutrients sufficient condition. On the contrary, a negligible PHB accumulation was found under the tested conditions, likely due to the high CO_2 concentration of 30 % *v/v* in the gas phase and therefore the high availability of inorganic carbon in the culture broth. This research demonstrated that *N. muscorum* could be a promising low-cost carbohydrate feedstock for bioethanol or bio-based chemicals production coupled to the removal of CO_2 from biogas or flue gas.

List of publications

Ángeles, R., Rodríguez, Á., Domínguez, C., García, J., Prádanos, P., Muñoz, R., Lebrero, R., 2020a. *Strategies for N_2 and O_2 removal during biogas upgrading in a pilot algal-bacterial photobioreactor*. Algal Research. https://doi.org/10.1016/j.algal.2020.101920

Ángeles, R., Arnaiz, E., Gutiérrez, J., Sepúlveda-Muñoz, C.A., Fernández-Ramos, O., Muñoz, R., Lebrero, R., 2020b. *Optimization of photosynthetic biogas upgrading in closed photobioreactors combined with algal biomass production*. Journal of Water Process Engineering 38, 101554. https://doi.org/10.1016/j.jwpe.2020.101554

Ángeles, R., Vega-Quiel, Miguel., Batista, Ariadna., Muñoz, R., Lebrero, R., 2021b. *Influence of biogas supply regime on photosynthetic biogas upgrading performance in an enclosed algal-bacterial photobioreactor* (accepted with minor revisions in Algal Research).

Ángeles, R., Arnaiz, E., Gutiérrez, J., Muñoz, R., Lebrero, R., 2021a. *Biogas-based production of glycogen by Nostoc muscorum: Assessing the potential of transforming CO_2 into value added products*. Chemosphere 275, 129885. https://doi.org/10.1016/j.chemosphere.2021.129885

Contribution to the papers included in the thesis

Paper I. In this research, I was responsible for the start-up, operation of the experimental set-up, and the evaluation of the results with the collaboration of Angel Rodriguez and Christian Domínguez under the supervision of Dra. Raquel Lebrero and Dr. Raúl Muñoz. I prepared the manuscript under the supervision of Dra. Raquel Lebrero and Dr. Raúl Muñoz. Dr. Pedro Prádanos and Dr. Juan García collaborated on the developed experimental design.

Paper II. In this work, I was responsible for the experimental methodology design, start-up, and operation of the experimental set-up with the collaboration of Julia Gutierrez under the supervision of Dra. Raquel Lebrero and Dr. Raúl Muñoz. I was responsible for the evaluation of the results and prepared the manuscript under the supervision of Dra. Raquel Lebrero and Dr. Raúl Muñoz. Oscar Fernandez-Ramos performed microalgal identification, where I contributed to the data analysis and discussion. Dra. Esther Arnáiz and Cristian A. Sepúlveda-Muñoz assisted in photobioreactor monitoring.

Paper III. During the execution of this research, I was responsible for the design, start-up, and operation of the experimental set-up, the evaluation of the results, and manuscript writing under the supervision of Dr. Raúl Muñoz and Dra. Raquel Lebrero. Miguel Vega assisted in photobioreactor monitoring. Dra. Ariadna Batista collaborated in the review and editing of the manuscript. Oscar Fernandez-Ramos performed microalgal identification, where I contributed to the data analysis and discussion.

Paper IV. In this work, I was responsible for the design, start-up, and operation of the experimental set-up, the evaluation of the results and manuscript writing under the supervision of Dra. Raquel Lebrero and Dr. Raúl Muñoz. Dra. Esther Arnáiz collaborated in the development of the analytical method for biopolymers analysis. Julia Gutierrez performed part of the batch assays monitoring.

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Chapter 1

</div>

Introduction

1.1 Biogas and biomethane production: a global perspective

Fossil fuels such as petroleum, coal, and natural gas constitute the main source for energy generation at a global scale. However, due to the increasing energy demand mediated by the increase in human population and industrialization, these reserves will be exhausted in the coming decades. Furthermore, most international political targets for climate change protection and energy production are based on reducing both greenhouse gas emissions and fossil fuels consumption, while promoting renewable energy generation (Sahota et al., 2018). In this context, renewable energy sources accounted for 8.4 % of the total energy generated worldwide in 2019, and the installed power capacity reached 2,588 GW (REN21, 2020). In the European Union (EU), a renewable energy contribution of 17 % to the total energy consumed was reported in 2015, with an installed power capacity of 402 Gigawatts (GW) (IRENA, 2020; REN21, 2016; Scarlat et al., 2018). Among renewable energy sources, biogas constitutes one of the main alternatives to reduce the current fossil fuel dependence and its associated environmental impacts. Thus, the global installed biogas capacity reached 19.5 GW in 2019, of which 13.5 GW corresponded to Europe, 2.7 GW to North America and 2.1 GW to Asia, Oceania and Africa as shown in Fig. 1.1 (IRENA, 2020). According to the Statistical Report of the European Biogas Association (EBA, 2018) published in December 2018, which covers 28 EU countries together with Iceland, Norway, Serbia and Switzerland, the total number of biogas plants in Europe increased by 2 % from 2016 to 2017, reaching 17,783 plants in 2017. Germany was the leader of biogas production in Europe with 10,971 plants, followed by Italy and France (1,655 and 742 plants, respectively). Overall, the Installed Electric Capacity (IEC) of biogas plants in Europe was 10,532 MW, which represented an increase of 5 % compared to 2016.

The anaerobic digestion of organic waste such as wastewater sludge, municipal organic waste or livestock manure, is a sustainable platform to simultaneously obtain renewable energy, reduce greenhouse gas emissions and treat organic waste (Muñoz et al., 2015; Sahota et al., 2018). Raw biogas produced by anaerobic digestion is primarily composed of methane (CH_4, 40 – 75 % v/v), carbon dioxide (CO_2, 15 – 60 % v/v), and trace levels of hydrogen sulphide (H_2S, 0.005 – 2 % v/v), oxygen (O_2, 0 – 2 % v/v), nitrogen (N_2, 0 – 3 % v/v), ammonia (NH_3, < 1 % v/v), carbon monoxide (CO, < 0.6 % v/v), hydrocarbons (VOC, < 0.6 % v/v), water vapor (5 –10

% v/v) and siloxanes (0 – 0.02 % v/v) (Ryckebosch et al., 2011). While some biogas contaminants such as CO_2 and N_2 decrease the calorific value of biogas, others such as H_2S and NH_3 are toxic and corrosive. The O_2 and N_2 present in biogas are not biologically generated during anaerobic digestion, although they are typically present at high concentrations in landfill gas when biogas is collected by vacuum generation as a result of air infiltration. Moreover, methylsiloxanes are highly detrimental for engines, microturbines or fuel cell power generators, and can also affect the post-combustion catalysts (Angelidaki et al., 2018).
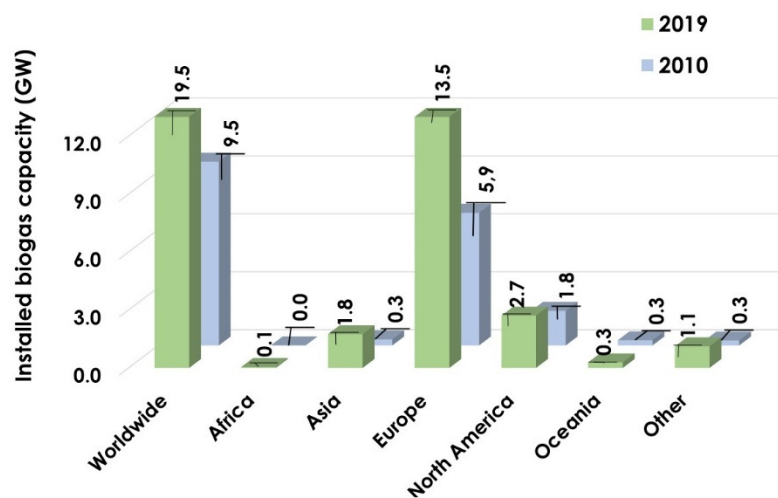
Fig. 1.1. Installed electric capacity at a global and regional scale (GW) (IRENA, 2020).

Biogas can be used to generate heat and electricity on-site in internal combustion engines (which requires H_2S abatement) or turbines (which requires a complete siloxane removal). However, if biogas is intended to be used as a transportation fuel or injected into natural gas grids, a stricter pretreatment is mandatory, including the reduction in CO_2, N_2, O_2, H_2O, H_2S, methyl-siloxanes and VOCs concentrations (see next section *Technical specifications for upgrading*). For this purpose, several biogas upgrading technologies such as pressure swing adsorption, scrubbing, membrane separation and cryogenic separation have been developed and commercialized at industrial scale, water scrubbing and membrane separation being nowadays the market-leading technologies worldwide (DMT-Environmental Technology, 2018; EBA, 2018).

Over the past few years, upgraded biogas (biomethane) has emerged as a promising renewable energy vector and, consequently, the European biomethane

sector has rapidly developed (Fig. 1.2). Biomethane production reached 2.28 billion m³ in the EU in 2018, whereas the number of biomethane plants installed in Europe has increased from 187 in 2011 to 660 in 2018 (EBA, 2019, 2018). In addition, the new European Renewable Energy Directive targets a contribution of 32 % for renewable energy by 2030, with sector-specific objectives that include annual increases of 1.3 % for renewable energy use in the heating sector and 14 % in the transportation sector by 2030. The "*Gas for Climate: a path to 2050*" initiative estimates that by 2050, the annual biomethane production could reach 1.072 TWh at a cost of 57 € MWh⁻¹, which would represent roughly 22 % of current natural gas consumption used in the industry, transportation and buildings and power generation (ECOFYS, 2018).
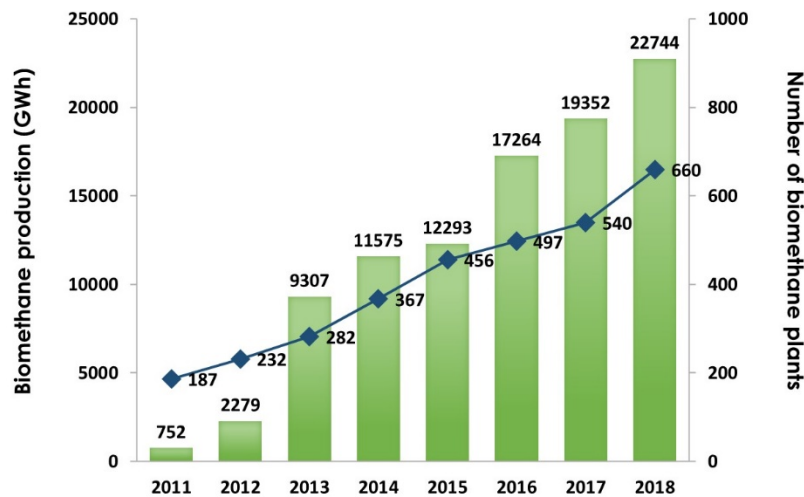
Fig. 1.2. Development of European biomethane production in GWh and number of biomethane plants (EBA, 2019, 2018).

Technical specifications for biogas upgrading

Based on the potential of biomethane as a renewable energy capable of substituting natural gas, manufacturers and countries have set standards for the utilization of the upgraded biogas as a fuel for stoves and boilers, engines and gas turbines, gas grid injection, substrate for fuel cells and use as a vehicle fuel (Allegue and Hinge, 2012). Thus, the required biomethane composition, and therefore the type of upgrading to be applied, depends on this final application (Table 1.1). Moreover, the selection of the most appropriate biogas upgrading technology should include factors such as investment and operating costs, recovery and loss of

methane, and removal efficiency for the biogas impurities above described (Bauer et al., 2013; Sun et al., 2015).

The use of biogas in boilers for heat generation only requires gas pressurization at 8 – 25 mbar, the reduction of water content and the removal of H_2S to a final concentration < 1000 ppm_v prior combustion. When biogas is to be used in domestic stoves the concentration of H_2S should be < 10 ppm_v (Allegue and Hinge, 2012; Petersson and Wellinger, 2009; Wheeler et al., 2000). H_2S concentration in biogas used for electricity generation in internal combustion engines should be reduced to 200 – 1000 ppm_v along with water in order to avoid the condensation of acid aqueous solutions in gas lines that could cause corrosion (Allegue and Hinge, 2012). Internal combustion engines also require levels of NH_3 < 32 – 50 mg m^{-3}, siloxanes < 5 – 28 mg m^{-3} and halocarbons < 65 – 100 mg m^{-3} prior to combustion. Moreover, the use of biogas for the combined production of heat and electricity on-site in turbines entails a maximum allowed H_2S concentration < 70,000 -10,000 ppm_v (depending on the size of the turbine), siloxanes < 0.03 – 0.1 ppm_v and halocarbons < 200 – 1500 ppm_v Cl^-/F^- (EPRI, 2006; Sun et al., 2015). Finally, fuel cells offer a high flexibility in terms of biogas composition due to their high operating temperatures (up to 1000 °C). Nevertheless, H_2S levels in biogas used as a substrate in fuel cells must be < 5 ppm_v and a complete siloxane removal is mandatory to prevent long-term damage in heat exchangers, catalysts and sensors (Haga et al., 2008).

On the other hand, when biomethane is intended to be used as a substitute of natural gas (i.e. for vehicle fueling or for injection into natural gas grids for further use in domestic gas appliances, cogeneration plants, or industry), technical specifications are stricter than those set by boilers, engines, or turbines manufacturers. Thus, an exhaustive purification is required in order to fulfill international biomethane standards, typically CH_4 ≥ 90 – 95 %, CO_2 ≤ 2 – 4 %, O_2 ≤ 1 %, H_2S < 5 – 15 mg m^{-3}, NH_3 < 3 – 20 mg m^{-3} and methylsiloxanes < 5 – 10 mg Si m^{-3} (Muñoz et al., 2015; Ryckebosch et al., 2011).

Table 1.1. Typical composition of different biogas and technical specifications for biomethane in Europe (Allegue and Hinge, 2012; Awe et al., 2017; Persson et al., 2006; Ryckebosch et al., 2011; Sun et al., 2015; Yang et al., 2014).

Parameter	Unit	Landfills gas	Biogas from anaerobic digestion	European Standardropean Standard EN 16723 – 1: 2016	Possible detrimental impact
Lower heating value	MJ Nm^{-3}	16	23	-	-
	kWh m^{-3}	4.4	6.5	-	
	MJ kg^{-1}	12.3	20.2	-	
Density	kg Nm^{-3}	1.3	1.2	-	-
Higher Wobbe index	MJ Nm^{-3}	18	27	-	-
CH$_4$ number		> 130	> 135	65	-
CH$_4$	% v/v	45	63	-	-
CH$_4$, variation	% v/v	36 – 65	53 – 70	-	-
H$_2$	% v/v	0 – 3	0	-	-
	% m/m			2	
CO	% v/v	0	0	0.1	-
CO$_2$	% v/v	40	47	2.5 or 4	Decreased calorific value, anti-knock properties of engines and corrosion
CO$_2$, variation	% v/v	15 – 50	30 – 47	-	-
N$_2$	% v/v	15	0.2	-	Decreased calorific value, anti-knock properties of engines, corrosion
N$_2$, variation	% v/v	5 – 40	-	-	-
O$_2$	% v/v	1	0	-	Corrosion, fooling in cavern storage, creation of explosive mixtures
	% mol			1	
O$_2$, variation	% v/v	0 – 5	-	-	-
H$_2$S	ppm$_v$	< 100	< 1000	-	Corrosion in compressor, gas storage tanks and engines. H$_2$S toxicity >5 cm^3 m^{-3} SO$_2$ and SO$_3$ formation during combustion: toxic and corrosive compounds in the presence of water
	mg m^{-3}			5	
H$_2$S, variation	ppm$_v$	0 – 100	0 – 1000	-	-
Sulphur	mg Nm^{-3}	-	-	-	Corrosion in the presence of water
NH$_3$	ppm$_v$	5	< 100	10	Corrosion in the presence of water
Si	mg Si m^{-3}	-	-	0.1 or 0.5	Erosion and corrosion of several equipment devices and clogging of pipes
Hydrocarbon Dew Point	°C	-	-	-2	
Water dew point	-	-	-	10	
Total chlorine (as Cl$^-$)	mg Nm^{-3}	20 – 200	0 – 5	-	Corrosion in combustion engines

Biogas upgrading technologies

Figure 1.3 shows the distribution of biogas upgrading technologies in Europe at industrial scale, a market dominated by physical-chemical technologies. In this sense, in 2017, from a total of 540 upgrading plants, 150 corresponded to water scrubbing, followed by membrane separation and chemical scrubbing (136 and 109 plants, respectively), pressure swing adsorption (70 plants), pressure swing absorption (25 plants) and cryogenic separation (10 plants) (EBA, 2018).
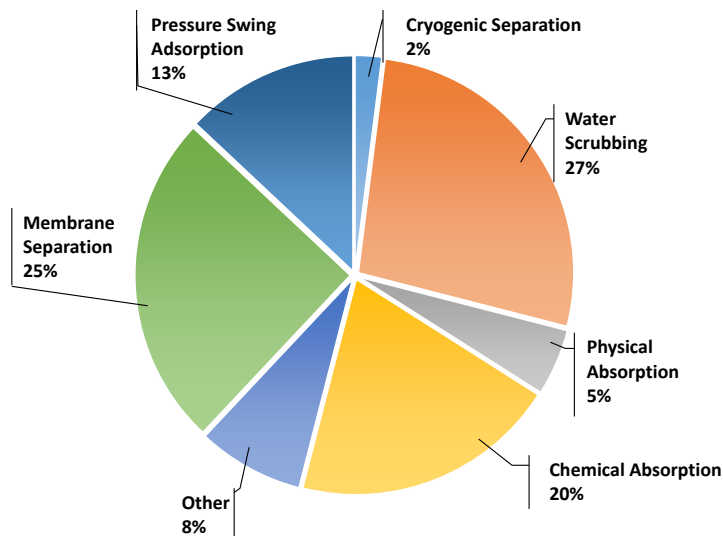

Fig. 1.3. Market share of biogas upgrading techniques in the EU in 2017 (EBA, 2018).

i. Physical-chemical technologies

Physical-chemical technologies for biogas upgrading are highly efficient and robust techniques, offering methane recoveries > 96 %, with a wide commercial availability (Table 1.2). However, these technologies present a high energy consumption, operating costs and chemical requirements, which limits the widespread exploitation of biomethane as a renewable energy source (Muñoz et al., 2015).

- *Physical and chemical absorption*

Water scrubbing is the most commonly implemented biogas upgrading technology, reaching up to 99 % of CH_4 purity in the upgraded biogas (Fig. 1.4a). This technology is based on the separation of CO_2 and H_2S from biogas due to their higher solubility in water compared to CH_4 (according to Henry's law, the solubility of CO_2 in water at 25 °C is approximately 26 times higher compared to that of CH_4)

(Angelidaki et al., 2018). The use of a chemical solution (e. g. alkanol amines or alkali aqueous solutions) boosts CO_2 gas-liquid mass transfer, thus reducing the required liquid recycling rates and the operating pressure in the absorption column, which also limits the losses of CH_4. H_2S is simultaneously absorbed in the amine scrubber (Angelidaki et al., 2018). Despite the high CH_4 content in biomethane (up to 99.8 %), the use of a toxic and corrosive chemical solution and the need for solvent regeneration in a stripping unit (Fig. 1.4b) results in potential environmental hazards, high energy requirements and increased investment and operating costs (Bauer et al., 2013; Haider et al., 2019; Ryckebosch et al., 2011).

- *Membrane separation*

Membrane separation relies on the selective permeability of biogas components across membranes under gas-gas (at high pressure) or gas-liquid configurations. The CH_4 content in the upgraded biogas can reach up to 98 % (Fig. 1.4c). Despite this high CH_4 recovery, this technology presents major disadvantages, such as the high cost of the membranes and the frequency of replacement (5-10 years), or the mandatory removal of H_2S, particles and siloxanes from raw biogas prior upgrading in order to avoid corrosion or clogging problems (Table 1.2) (Bauer et al., 2013; Persson et al., 2007).

- *Pressure swing adsorption*

Pressure swing adsorption (PSA) is based on the different affinity of biogas contaminants to certain adsorbents, typically activated carbon, zeolite, activated alumina or silica gel, allowing their selective retention in a vertical packed column working at high pressure. Conventional PSA units consist of 4 interconnected columns for successive pressurization, adsorption at 4-10 bar, depressurization and desorption for adsorbent regeneration (Fig. 1.4d) (Awe et al., 2017). Unfortunately, the adsorption of H_2S is irreversible, and therefore its removal is necessary prior injection of the biogas in the PSA column (Angelidaki et al., 2018). This technology is simple in terms of operation, and exhibits a moderate energy use and capital investment (Table 1.2) (Augelletti et al., 2017).

- *Cryogenic separation*

Cryogenic separation can remove CO_2, N_2 and O_2 from the raw biogas due to the different condensing temperatures and vapor pressures. Biogas is initially dried and compressed up to 80 bars and gradually cooled down to temperatures of $-110\ °C$.

This gradual temperature decrease entails the condensation of H_2O, siloxanes, VOCs and CO_2, which ultimately results in a CH_4 content > 97 % in the upgraded biomethane (Ryckebosch et al., 2011; Sun et al., 2015). However, cryogenic CO_2 separation is still in an embryonic stage, presenting high investment and operating costs, and with few facilities in operation at commercial scale. CH_4 losses and clogging problems derived from CO_2 solidification rank among the main operational problems of this technology (Bauer et al., 2013; Muñoz et al., 2015).

Table 1.2. Comparison of different pilot and commercial physical-chemical biogas upgrading technologies (Angelidaki et al., 2018).

|	Cryogenic separation	PSA	Water scrubbing	Organic scrubbing	Chemical absorption	Membrane separation
Electricity consumption (kWh Nm^{-3} raw biogas)	0.8	0.2 – 0.3	0.25 – 0.3	0.2 – 0.3	0.1 – 0.2	0.18 – 0.2
Electricity consumption (kWh Nm^{-3} clean biogas)	NF	0.3 – 1.0	0.3 – 0.9	0.4	0.1 – 0.3	0.1 – 0.3
Heat consumption (kWh Nm^{-3} raw biogas)	NF	None	None	> 0.2	0.5 – 0.75	None
Temperature limit (°C)	-196	-	-	55 – 80	100 – 180	-
Cost	High	Medium	Medium	Medium	High	High
CH$_4$ losses (% v/v)	2	< 4	< 2	2 – 4	< 0.1	< 0.6
CH$_4$ recovery (% v/v)	97 – 98	96 – 98	96 – 98	96 – 98	96 – 99	96 – 98
Pre-purification	Yes	Yes	Suggested	Suggested	Yes	Suggested
H$_2$S co-removal	Yes	Possible	Yes	Possible	Pollutant	Possible
N$_2$ and O$_2$ co-removal	Yes	Possible	No	No	No	Partial
Operation pressure (bar)	80	3 – 10	4 – 10	4 – 8	Atmospheric	5 – 8
Outlet pressure (bar)	8 – 10	4 – 5	7 – 10	1.3 – 7.5	4 – 5	4 – 6

NF: not found.
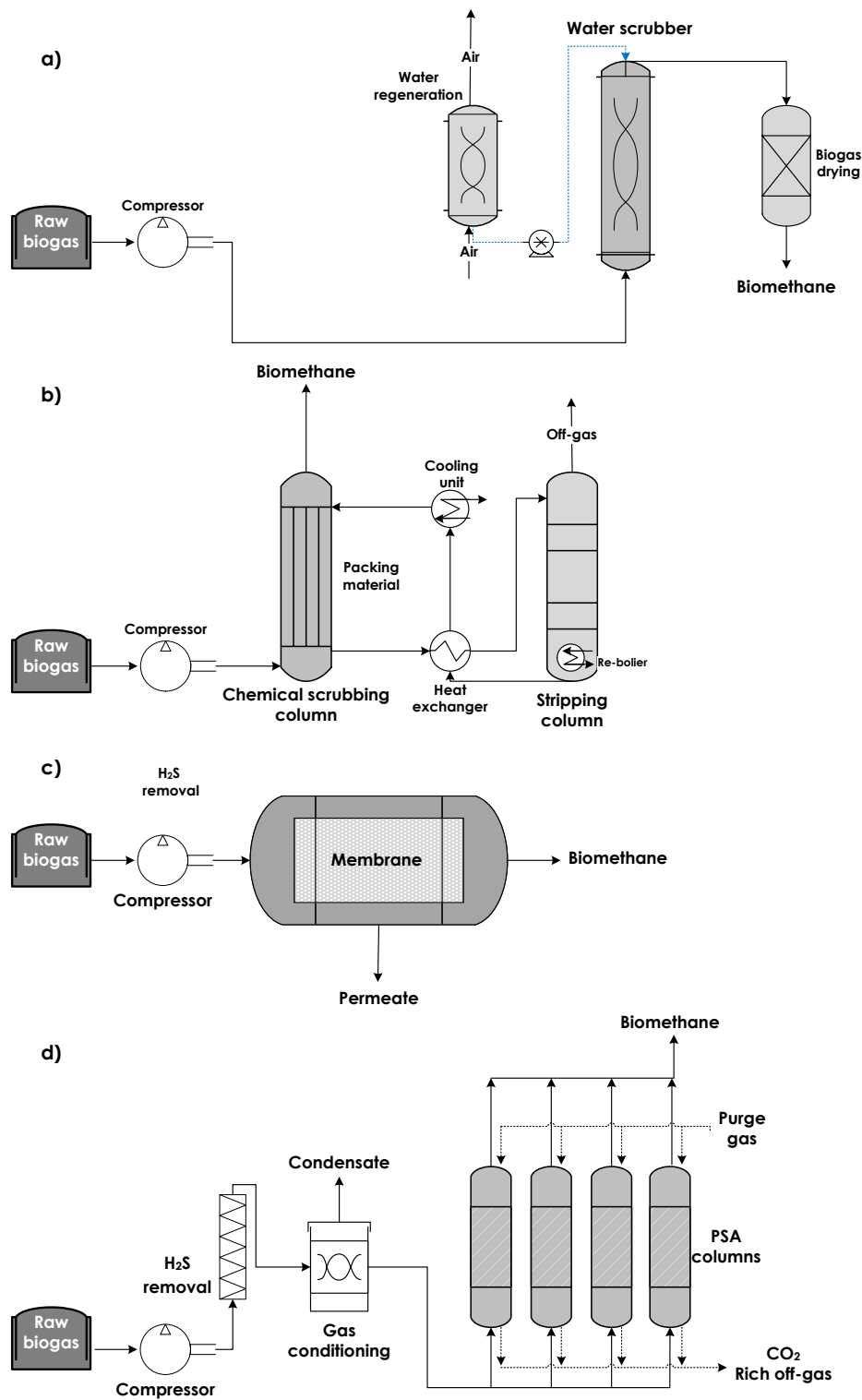
Fig. 1.4. Physical and chemical biogas upgrading technologies; a) water scrubbing with water regeneration, b) chemical absorption (amine scrubbing), c) membrane system (general design), and d) pressure swing adsorption. Adapted from Angelidaki et al., (2019); Kapoor et al., (2019).

ii. Biological technologies

Biological biogas upgrading based on chemoautotrophic and photosynthetic microorganisms has attracted significant attention in the past years. These technologies, which *a priori* present lower operating costs and environmental impacts, can convert CO_2 into CH_4 or value added microalgal biomass, respectively. Extensive research has been recently conducted for their optimization at laboratory scale, most of them being currently under validation at pilot scale (Angelidaki et al., 2018).

- ### *Hydrogenotrophic CO_2 removal*

Hydrogenotrophic biogas upgrading is based on the bioconversion of the CO_2 contained in the biogas into CH_4 by hydrogenotrophic methanogenic archaea, using H_2 as an electron donor (Eq. 1) (Muñoz et al., 2015).

$$4H_2 + CO_2 \rightarrow CH_4 + H_2O \quad \Delta G^0 = -130.7 \: KJ \: mol^{-1}$$ **(Eq. 1)**
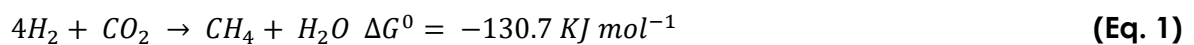
The H_2 required for the conversion of CO_2 should be supplemented from a renewable energy source in order to ensure the environmental sustainability of this technology. For instance, the electricity surplus generated by wind turbines or solar panels can be used to electrolyze water into H_2 and O_2 (Angelidaki et al., 2019).

Chemoautotrophic biogas upgrading has been validated at laboratory and pilot scales in three different configurations: (i) *in-situ* biogas upgrading, via direct H_2 injection into the anaerobic digester, (ii) *ex-situ* biogas upgrading, using an external bioreactor for CO_2 conversion, and (iii) a hybrid process integrating both in-*situ* and *ex-situ* configurations (Goleman et al., 2017). This biological biogas upgrading technology is mainly limited by the low aqueous solubility of H_2, which decreases gas-liquid mass transfer and therefore H_2 availability for methanogens.

- ### *Photoautotrophic CO_2 removal*

Photosynthetic biogas upgrading in algal-bacterial photobioreactors (PBRs) constitutes an alternative for the simultaneous removal of CO_2 and H_2S from raw biogas. This technology is based on CO_2 fixation by microalgae via photosynthesis and the concomitant H_2S oxidation by sulphur-oxidizing bacteria utilizing the oxygen

photosynthetically produced (Bahr et al., 2014). The potential of this innovative technology has been demonstrated both indoors and outdoors in pilot and demo high rate algal ponds (HRAPs) interconnected to biogas scrubbing columns (SCs) (Bahr et al., 2014; Posadas et al., 2015a; Rodero et al., 2018a; Serejo et al., 2015; Toledo-Cervantes et al., 2017b). The fundamentals of this technology are further presented and discussed in section 1.2.

1.2 Photosynthetic biogas upgrading

Fundamentals of photosynthetic biogas upgrading

Photosynthetic microorganisms such as cyanobacteria and microalgae fix CO_2 via photosynthetic carboxylation reactions (Calvin-Benson-Bassham (CBB) cycle, Fig. 1.5), which takes place in the plasmalemma membrane in cyanobacteria or in the chloroplasts in microalgae. The light energy is absorbed as photons and converted to adenosine triphosphate (ATP) into the internal membrane of the chloroplasts, in the thylakoids and branches of the plasma membrane, where photosynthetic pigments (chlorophylls, carotenoids, and in some cases phycobilins) and the enzymes necessary for the use of light and fixation of CO_2 are found. The energy is used by photosystem II (PSII) for the oxidation of water, liberating protons, electrons, and molecules of oxygen, a process known as Hill reaction. Afterwards, the products from the light reaction (i.e. nicotinamide adenine dinucleotide phosphate (NADPH) and ATP) are used as substrates for the light-independent phase. In this phase, CO_2 is incorporated into ribulose–1,5–bisphosphate (RuBP) catalyzed by the ribulose–1,5–biphosphate carboxylase/oxygenase (RuBisCO) enzyme, resulting in two molecules of 3–phosphoglycerate (3–PG). The final product of CO_2-fixation is glyceraldehyde–3–phosphate (G3P) via phosphorylation and reduction of 3–PG (Klemenčič et al., 2017; Severo et al., 2019).

On the other hand, sulphide-oxidizing bacteria create a symbiotic relationship with microalgae for the biological oxidation of H_2S using the high dissolved oxygen (DO) concentration from photosynthetic activity (Eq. 2) (Fig. 1.6) (Muñoz et al., 2015).
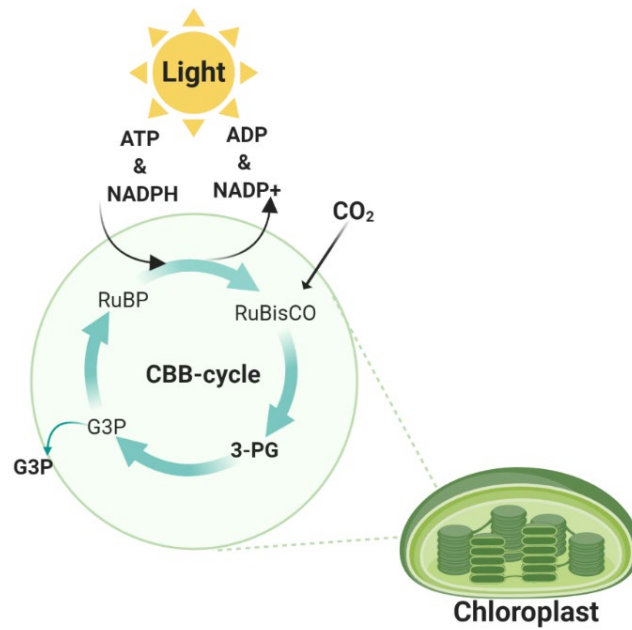
$$H_2S \ + \ 2O_2 \ \rightarrow \ SO_4^{2-} \ + \ 2H^+ \hspace{4cm} \textbf{(Eq. 2)}$$

Fig. 1.5. Schematic of the photosynthetic process in chloroplasts and the Calvin-Benson-Bassham (CBB) cycle for CO_2 fixation. Abbreviations: ADP, adenosine-diphosphate; ATP, adenosine-triphosphate; NADP+, nicotinamide adenine dinucleotide phosphate (oxidised); NADPH, nicotin-amide adenine dinucleotide phosphate (reduced); RuBP, ribulose–1,5–bisphosphate; RuBisCO, ribulose–1,5–biphosphate carboxylase/oxygenase;3-PG, 3–phosphoglycerate; G3P, glyceraldehyde–3–phosphate. (Figure created with BioRender.com).
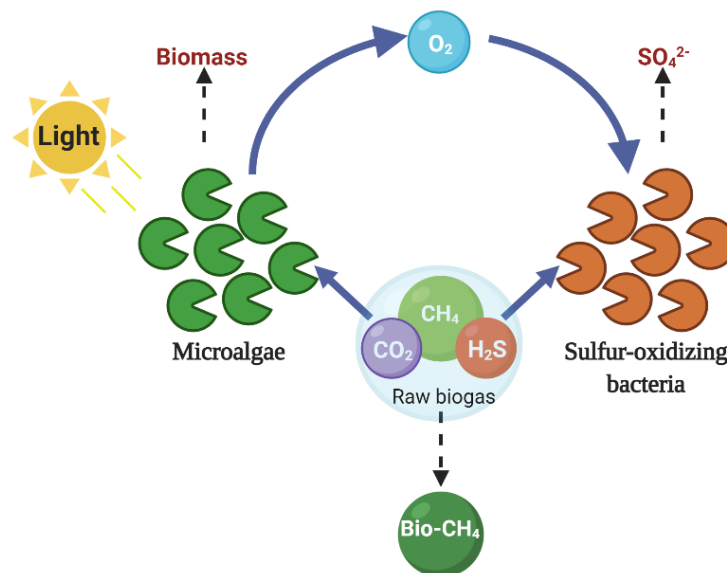

Fig. 1.6. Symbiotic mechanisms during photosynthetic biogas upgrading in microalgae-bacteria PBRs. (Figure created with BioRender.com).

Implementation of photosynthetic biogas upgrading

Prior biological conversion, CO_2 and H_2S must be transferred from the raw biogas to the aqueous cultivation broth. To this aim, the photobioreactor, commonly a HRAP, is interconnected to an external scrubbing column where the raw biogas is fed. In order to control algal biomass concentration and productivity, a settler is typically implemented between the HRAP and the SC. Finally, the nutrients needed for biomass growth can be supplemented by a waste stream (i.e. centrifuged digestate from the anaerobic digestion process) (Fig. 1.7). This configuration has been validated and optimized both indoors and outdoors in pilot and demo plants, achieving biomethane composition of $CH_4 > 64$ %, CO_2 $0.9 - 9$ %, and $N_2 + O_2$ $0 - 22$ %, together with biomass productivities of up to 22.5 g m^{-2} d^{-1} (Table 1.3)(Bahr et al., 2014; Meier et al., 2017, 2019; Posadas et al., 2017b; Rodero et al., 2019; Serejo et al., 2015).
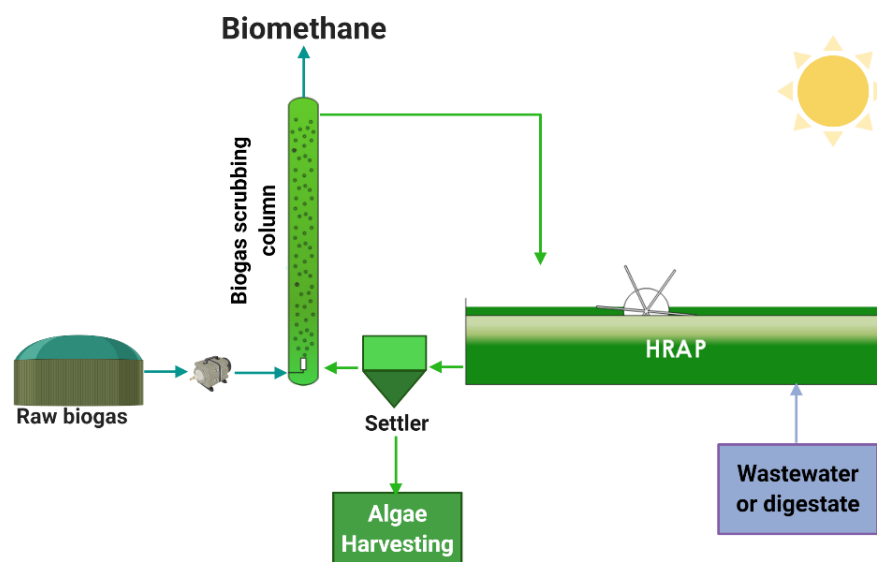
Fig. 1.7. Schematic representation of a photosynthetic biogas upgrading system consisting of a HRAP interconnected to a biogas SC. (Figure created with BioRender.com).

Parameters affecting biogas upgrading

Despite the satisfactory CO_2 removal efficiencies > 90 % and an almost complete H_2S abatement, preliminary experiments did not result in a biomethane composition complying with most biomethane standards due to the contamination of the upgraded biogas with N_2 and O_2 or the presence of high concentrations of CO_2. Among the main design, operational and environmental parameters, the liquid to

biogas flowrate (L/G) ratio and the operating pressure in the SC, the alkalinity and the pH of the cultivation broth, the microalgae species, the dissolved O_2 and N_2 concentration, and the type of photobioreactor configuration are those having the greatest influence on the performance of photosynthetic biogas upgrading (Bose et al., 2019; Rodero et al., 2018a; Srinuanpan et al., 2020).

- ***Illumination***

Light is the most important factor influencing microalgae growth since it provides the energy required to convert dissolved inorganic carbon into biomass via photosynthesis (Posadas et al., 2017a). Approximately 1 mol of oxygen is released per mol of captured CO_2 through oxygenic photosynthesis (Meier et al., 2017). In this way, during the illuminated period of photosynthetic biogas upgrading, the high DO concentration resulting from photosynthetic activity triggers an increase in the pH of the cultivation broth and a rapid sulphate oxidation in the presence of sulphur-oxidizing bacteria (Franco-Morgado et al., 2017). On the contrary, in the absence of light, the stoppage of photosynthesis leads to a decrease in the DO concentration, which could affect the oxidation capacity of H_2S. Thus, if biogas is supplemented during the dark period, inhibition of microalgae growth derived from H_2S accumulation might occur, while CO_2 accumulation could decrease the pH to levels preventing an effective CO_2 capture. However, the accumulation of NO_3^- during the light period in a PBR (produced from the oxidation of the NH_4^+ supplemented when using wastewater as a nutrient source) could support the anoxic oxidation of H_2S during the dark period, thus preventing H_2S induced inhibition.

- ***Operating parameters of the scrubbing column***
    - <u>*Liquid to biogas flowrate ratio*</u>

The external biogas SC coupled to the photobioreactor is commonly operated under co-current flow at a superficial gas and liquid velocities below 4 cm s^{-1} (Bose et al., 2019). Despite the higher overall concentration gradients and mass transfer rates resulting from counter-current operation in the absorption column, the increased O_2 and N_2 stripping together with the accumulation of sulphur in the

biogas diffuser due to the depletion of oxygen at the bottom of the column makes co-current operation the preferred configuration during photosynthetic biogas upgrading. The L/G ratio in the biogas SC must be also optimized in order to provide a satisfactory biomethane quality. L/G ratios < 1 usually entail a significant increase in CO_2 and H_2S removal along with a lower O_2 and N_2 content in the upgraded biomethane (Toledo-Cervantes et al., 2017b).

Operating pressure

Despite the high CO_2 and H_2S removal efficiencies achieved in photosynthetic biogas upgrading, further improvements should focus on the reduction of N_2 and O_2 concentration in the upgraded biogas in order to comply with most biomethane standards. In this regard, the use of an external pressurized SC could minimize the desorption of N_2 and O_2 from the cultivation broth to the biomethane. An increase in the operating pressure of the SC will increase the solubility of these gases in the liquid phase, thus minimizing their desorption to the upgraded biomethane. Besides, it will also increase the CO_2 gas-liquid gradient, enhancing the mass transfer of this contaminant from the biogas to the cultivation broth. By contrast, a higher operating pressure could also result in a slightly higher CH_4 solubilization (Bahr et al., 2014; Serejo et al., 2015).

- ### *Operating parameters of the cultivation broth*
  #### pH

Several microbiological and mass transfer processes are affected by the pH of the cultivation broth during photosynthetic biogas upgrading coupled with wastewater treatment: the activity and growth of the microalgae, the removal of nutrients from the liquid waste stream, and the mass transfer of CO_2 and H_2S from the biogas into the cultivation broth in the SC. CO_2 absorption in the alkaline solution of the SC takes place according to Eq. 3, bicarbonate being then incorporated within the cells as CO_2 by the action of the enzyme carbonic anhydrase (CA) (Eq. 4), which subsequently increases the pH and allows carbonate regeneration (Eq. 5). The formation of bicarbonate by CO_2 absorption and subsequent regeneration of

carbonate by algal photosynthesis results in both natural maintenance of the carbonate/bicarbonate cycle and pH regulation (Bose et al., 2019).

$$CO_2 + H_2O + CO_3^{2-} \rightarrow 2HCO_3^-$$ **(Eq. 3)**

$$HCO_3^- \xrightarrow{CA} CO_2 + OH^-$$ **(Eq. 4)**

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$$ **(Eq. 5)**

Previous studies have demonstrated that CO_2 removal is significantly improved at pH >9 regardless of the L/G ratio, reaching CO_2 removal efficiencies up to 98 %. This enhancement is associated to the increase in the solubility of CO_2 at a high pH, finally resulting in an increase in CO_2 mass transfer from the biogas. Likewise, H_2S is an acid gas, thus its absorption into the aqueous solution will be also promoted at high pH values (Franco-Morgado et al., 2017; Rodero et al., 2018a; Serejo et al., 2015; Toledo-Cervantes et al., 2017b).

Alkalinity

The alkalinity of the cultivation broth has a positive effect on the removal of acidic gases such as CO_2 and H_2S. A high alkalinity results in a higher buffer capacity and a lower decrease in pH along the SC. Thus, higher alkalinities promote the aqueous solubility of both acidic gases and enhance mass transfer rates between the biogas and the liquid phase. Previous studies have demonstrated the key role of this parameter in biomethane quality, reaching up to 98 % CH_4 content at high inorganic carbon concentrations (IC > 1500 mg L^{-1}) (Rodero et al., 2018a).

- ### **Microalgae population structure**

Microalgae/cyanobacteria from the genera _Chlorella, Pseudanabaena, Scenedesmus,_ and _Spirulina_ have been identified as the most common microorganisms during photosynthetic biogas upgrading (Table 1.3). These microalgae/cyanobacteria have the ability to grow mixotrophically, besides showing a high tolerance to pH and CO_2 concentration (Angelidaki et al., 2019; Bose et al., 2019). In particular, mixotrophic cultures (using both inorganic and

organic carbon) offer an additional advantage during the treatment of wastewater or anaerobic digestates.

- ***Degassing of the scrubbing solution***

N_2 and O_2 desorbed from the cultivation broth in the SC is an important factor limiting biomethane applications. Hence, the selective separation of these gases from the cultivation broth before entering the SC will reduce N_2 and O_2 concentrations in the upgraded biogas. In this context, the degasification of the recirculating stream using a polydimethylsiloxane (PDMS) gas-liquid membrane with higher permeability for N_2 and O_2 located between the settler and the SC will likely result in a higher biomethane quality.

- ***Photobioreactor configuration***

Microalgae cultivation has been traditionally performed in open (circular or high-rate algal ponds) and enclosed (tubular, column, or flat paneled) PBRs with either artificial or natural light (Fig. 1.8). Key factors in the design of PBRs for biogas upgrading and microalgae-based wastewater treatment are a large surface area, efficient light supply with short internal light paths, enhanced gas exchange, and efficient mixing (Carvalho et al., 2006; Eriksen, 2008). The PBR footprint must be also taken into account in land-restricted sites. Additionally, contamination control becomes a crucial parameter in PBRs devoted to biomass revalorization into high value added products. However, in the context of biogas upgrading, the high pH and alkalinity of the cultivation broth typically result in a low algal diversity and a lower risk of contamination.

Open ponds

Open ponds are the most common configuration for large-scale microalgae cultivation. The widespread use of open ponds is mainly attributed to their simple design, construction, and operation, their low energy requirements, and therefore the lower associated costs. On the contrary, open ponds present large space requirement, a low photosynthetic efficiency mediating a low biomass density, a poor gas-liquid mass transfer (due to the limited contact time gas–culture), and a limited control over environmental parameters. Moreover, the exposure of the

culture to the open atmosphere facilitates microbial contamination and water evaporation (Chang et al., 2017; Chisti, 2007; Posten, 2009).

Fig. 1.8. HRAP (a) and tubular (b) PBRs located at the facilities of the Institute of Sustainable Processes, Valladolid University (Spain).

Circular ponds (Fig. 1.9b) are one of the traditional pond configurations used for commercial algae cultivation, particularly suited for easily settleable biomass. They are commonly constructed in concrete and lined with materials (i.e., plastic sheets or inert membranes). A rotating arm is placed in the center of the pond to provide better mixing of the cultivation broth (Borowitzka and Moheimani, 2013).

However, more than 95 % of the algae production worldwide is cultivated in HRAPs (also named raceway ponds), the most popular open PBR used at commercial scale due to their flexibility and easy scale-up (Fig. 1.9a). In HRAPs, the culture broth containing the microalgae, water, and nutrients is continuously recirculated around a racetrack consisting of two or four parallel channels (Benemann, 2013; Costache et al., 2013; Posten, 2009). Water channel depth is limited to improve light penetration: the lower the water layer, the higher the light penetration, biomass concentration, and culture stability (Acién et al., 2017; Singh and Sharma, 2012). They are equipped with a paddle wheel that provides a continuous, slow, and non-turbulent mixing, preventing sedimentation and reducing cell damage induced by shear stress (Fig. 1.9a).

Nevertheless, this mild mixing might also result in flocculation of the cells, impeding proper light penetration and leading to lower biomass productivities (Ugwu and

Aoyagi, 2012). In order to increase the gas-liquid contact time and overcoming poor CO_2 mass transfer efficiency, raceway ponds are usually endowed with sumps or interconnected to external absorption columns (Park et al., 2011; Posadas et al., 2017b). Typical design parameters are hydraulic retention time (HRT) of 2 – 8 days, total surface area ranging from 100 up to 10,000 m^2, water depths from 20 to 40 cm, and length-to-width (L/W) and surface-to-volume (S/V) ratios of 10 – 20 and 5 – 10 m^{-1}, respectively. Further information on the design criteria and power consumption calculations can be found elsewhere (Acién et al., 2017; Posadas et al., 2017b, 2017a).

Microalgae species such as *Chlorella* sp., *Dunaliella* sp., or *Spirulina* sp. are commonly cultivated in HRAPs due to their tolerance to high pH and salinities that might inhibit the growth of other algae or pathogenic bacteria (Ugwu and Aoyagi, 2012). When HRAPs are used to treat wastewater, microalgae from the genera *Chlorella* or *Scenedesmus* tend to dominate the cultivation broth based on their tolerance to organic pollution.
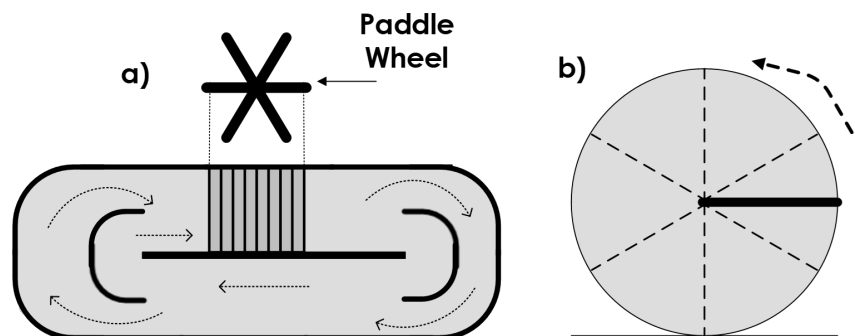

Fig. 1.9. Schematic representation of most typical open pond configurations: (a) HRAPs and (b) circular pond.

Enclosed photobioreactors

Enclosed PBRs overcome the main disadvantages encountered in open systems, allowing for better control of environmental parameters such as temperature, pH, light, and CO_2 concentration, minimizing CO_2 losses and water evaporation and preventing culture contamination. This last advantage is of key importance when targeting the production of high-quality, complex bioproducts since they facilitate the cultivation of single, pure algal strains. They also provide large S/V ratios, shortening internal light paths, and improving photosynthetic efficiency (Carvalho

et al., 2006; Chang et al., 2017; Janssen et al., 2003). The high internal circulation velocities (up to 1 m s^{-1}) of the cultivation broth offer an efficient mixing and boost gas-liquid mass transfer but limit their application to microalgal strains tolerant to shear stress. On the contrary, enclosed systems are limited by the poor settling ability of the biomass, potential biomass washout, and harvesting limitation. Moreover, the dissolved oxygen concentration in the PBR might reach inhibitory levels for microalgae and contaminate biomethane if the system is operated at long HRT or low organic loading rates (Costache et al., 2013; Molina et al., 2001). The absence of continuous gas exchange with the atmosphere has typically limited biogas upgrading in enclosed PBRs to process operation during the illuminated period in order to prevent the inhibitory accumulation of H$_2$S.

Tubular PBRs are the most commonly implemented enclosed PBR configuration at industrial scale for microalgae cultivation (i.e., 25 m^3 reactors operated by Mera Pharmaceuticals in Hawaii or the 700 m^3 plant in Klötze, Germany (Eriksen, 2008)) (Fig. 1.18b). In tubular PBRs, microalgal culture flows through long, transparent tubing of < 0.1 m of inner diameter arranged either horizontally, vertically, inclined, or helically. The cultivation broth is recirculated by mechanical pumping, a paddle wheel or aeration to provide turbulence and prevent biomass settling in the tubes (Acién Fernández et al., 2013; Carvalho et al., 2006; Marín et al., 2019). Tubular PBRs present S/V ratios of ~80 m^{-1}, the length and diameter of the tubes being key design parameters that determine both the dissolved O$_2$ accumulation and head loss. Power requirement in tubular PBRs ranges from 10 to 100 W m^{-2}, and the liquid velocity through the tubes is usually set between 0.1 and 0.8 m s^{-1} in order to minimize power consumption and avoid cell damage. If aeration is used, aeration rates of 0.01 – 0.10 v v^{-1} min^{-1} are selected, the mass transfer coefficient depending on the type of diffuser and gas flow rate (Acién et al., 2017). Additional information on the design parameters of tubular PBRs can be found elsewhere (Acién et al., 2017; Carvalho et al., 2006).

Vertical tubular PBRs are compact, easy to operate reactors with a high S/V ratio, low contamination risk, and high biomass productivity, thus suitable for large-scale cultivation of microalgae (Chang et al., 2017). Their enclosed nature prevents the constant intrusion of N$_2$ from the atmosphere, which will ultimately limit N$_2$ desorption and contamination of the biomethane. Depending on the type of mixing, they can be classified into stirred tank PBRs, bubble columns, or air-lift PBRs (Acién Fernández

et al., 2013) (Fig. 1.10). Stirred tank reactors use mechanical agitation through one or several impellers, the CO_2 being supplied by CO_2-enriched air bubbling from the bottom of the reactor (Fig. 1.10a). Although this configuration has been applied for the production of high-value products because of the superior control over process parameters and microbial contamination, insufficient illumination for the photosynthetic microalgal activity usually hinders their implementation at large scale (Acién Fernández et al., 2013; Carvalho et al., 2006; Singh and Sharma, 2012).

Bubble column PBRs are vertical cylindrical aerated columns that provide a high homogeneity of the culture broth conditions, an improved mass and heat transfer, and an efficient oxygen stripping (Fig. 1.10b). However, photosynthetic efficiency greatly depends on the gas flow rate, since the erratic turbulence created by gas sparging might result in uneven exposure of microalgal cells to light intensity, and sedimentation is more likely to occur (Chang et al., 2017; Singh and Sharma, 2012).

Finally, air-lift PBRs consist of two interconnected zones: the riser, where the gas is sparged, and the internal downcomer, the region that does not receive gas but drives liquid recirculation. Most common configurations used for air-lift PBRs are internal loop (Fig. 1.10c), internal loop concentric (Fig. 1.10d), and external loop vessels (Fig. 1.10d), the latter offering an enhanced mixing because of the distance between the riser and the downcomer. The presence of the two zones generates a circular and homogeneous mixing pattern, where the liquid culture moves continuously through dark and light zones. The residence time of the culture broth in each zone will affect light uptake, heat transfer, mass transfer, mixing, and turbulence (Chang et al., 2017; Chisti and Moo-Young, 1993; Degen et al., 2001; Xu et al., 2009). Interestingly, due to the characteristic mixing pattern of air-lift PBRs, higher growth rates are achieved in this PBR configuration compared with those obtained in bubble column PBRs (Xu et al., 2009).
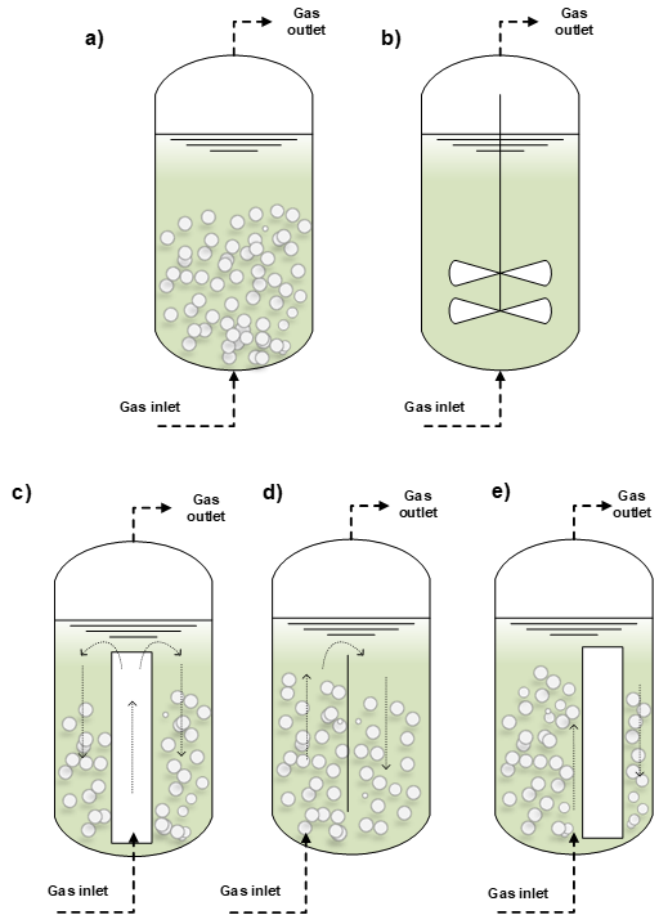
Fig. 1.10 Schematic representation of typical vertical tubular PBR configurations: (a) bubble column, (b) stirred tank, (c) internal loop split airlift, (d) internal loop concentric tube airlift, (e) external loop vessels airlift. (Adapted from Carvalho et al., (2006); Massart et al., (2014)).

Table 1.3. Experimental studies on photosynthetic biogas upgrading.

Photobioreactor configuration	L/G	IC (mg L^{-1})	pH	Microalgae species	Raw biogas composition (% v/v)			Biomethane composition (% v/v)			CO$_2$-RE (%)	H$_2$S-RE (%)	Biomass concentration (g L^{-1})	Reference
					CH$_4$	CO$_2$	H$_2$S	CH$_4$	N$_2$	O$_2$				
HRAP (indoors)	10	142	7.9	*Microspora* sp. *Planktolyngbya* sp. *Geitlerinema* sp.	70	29.5	0.5	80	< 9	< 2	80	100	0.49	Serejo et al., (2015)
HRAP (indoors)	10	-	8	*Geitlerinema* sp. *Pseudanabaena minima Limnothrix planktonica*	70	29.5	0.5	81	7	< 1.2	72 – 79	100	1.07	Posadas et al., (2015a)
Enclosed PBR (indoors)	-	-	< 11	*Scenedesmus* sp.	~70	~22	1200 – 1900*	64	-	22	66	99	1.1	Prandini et al., (2016)
HRAP (indoors)	1	4450	10	*Geitlerinema* sp. *Staurosira* sp. *Stigeoclonium tenue*	70	29.5	0.5	> 95	0.2	0.03	> 98	> 97	-	Toledo-Cervantes et al., (2016)
HRAP (indoors)	5	2400	9.7	*Picochlorum* sp. *Halospirulina* sp.	-	30	0.5	-	-	2.6	94	99.5	0.23	Franco-Morgado et al., (2017)
HRAP (indoors)	0.5	1500	10.5	*Muchonastes homosphaera*	70	29.5	0.5	96	2.7	0.7	97	100	2.6	Toledo-Cervantes et al., (2017b)
HRAP (outdoors)	0 – 5	2000	10	*Pseudanabaena* sp.	70	29.5	0.5	85	1 – 3	0.1 – 2.0	86	100	0.6	Posadas et al., (2017b)
HRAP (indoors)	0.6	-	7	*C. sorokiniana*	65	32	-	-	-	< 1	89 – 93	-	0.6	Meier et al., (2017)
HRAP (indoors)	0.5	1500	11	-	70	29.5	0.5	98	0.7	< 0.2	> 97	> 96	0.43 – 0.54	Rodero et al., (2018a)
HRAP (outdoors at semi-industrial scale)	3.5	500	8.9	-	69.2	32.7	1183*	90	11.4		99	100	0.56	Rodero et al., (2019)

*ppm$_v$ H$_2$S

Operating parameters in algal biomass cultivation

Environmental parameters (i.e. pH, dissolved oxygen, temperature, irradiation, and water evaporation rate), HRT, mixing and gas-liquid mass transfer represent the main operating parameters influencing microalgae cultivation (and wastewater treatment) in the above described photobioreactor configurations.

- *Hydraulic Retention Time*

The average period of time that the wastewater remains in the PBR is given by the HRT. Therefore, both carbon and nutrient load supplied to the PBR, and consequently the biomass productivity, are determined by the HRT (Arbib et al., 2013; Metcalf & Eddy, 2003). The HRT can be calculated according to Equation 6:

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

Where V is the PBR volume (m^3) and Q the influent wastewater flow rate ($m^3\ d^{-1}$). HRTs between 2 and 50 days are commonly required for an efficient removal of both organic pollutants and nutrients, this value depending on the characteristics of the wastewater, the PBR configuration, and the environmental conditions. In this sense, enclosed systems usually require lower HRTs (2 – 5 days) compared with open PBRs (i.e., 4 – 9 days for HRAPs)during the treatment of domestic wastewater (Luo et al., 2017; Muñoz and Guieysse, 2006). The use of digestate, common strategy during photosynthetic biogas upgrading, requires HRT ranging from 25 to 75 days depending on the concentrations of N and P.

Biomass productivity is expected to decrease due to biomass washout when the HRT is lower than the specific growth rate (μ). Ruiz et al., (2013) observed maximum biomass production and CO_2 biofixation at a HRT of 2 d^{-1}, whereas HRT close to μ^{-1} allowed for an optimum nutrient removal. On the other hand, the use of digestate to support algal growth require the engineering of innovative process configuration to prevent an excessive alga biomass concentration. This technological limitation during photosynthetic biogas upgrading was overcome by implementing a settler between the SC and the PBR, which allowed controlling biomass productivity.

- ***Gas-Liquid Mass Transfer***

Carbon, the major substrate for photoautotrophic microalgal growth, is mainly provided to the microalgal culture in the form of CO_2 via bacterial respiration of organic matter during wastewater treatment or external CO_2 supply in absorption units. Carbon fixation from the atmospheric CO_2 is not efficient due to its low concentration (~0.03 %) and reduced solubility. In this context, sparging a CO_2-laden stream (such as flue gas or biogas) in the cultivation broth would improve biomass productivity and therefore boost nutrients assimilation (Pires et al., 2017). Thus, increasing the CO_2 mass transfer from the gas to the cultivation medium is necessary to increase the total inorganic carbon available for microalgae growth (Chang et al., 2017). At this point, it is worth noting that the k_La can be affected by numerous factors such as the agitation, the gas flowrate, gas pressure, temperature, reactor geometry, properties of the fluid (density, viscosity), or presence of antifoaming agents in the cultivation broth in the SC (Barbosa et al., 2003; Janssen et al., 2003). Different strategies such as improving culture mixing or implementing gas recirculation have been proposed for overcoming gas-liquid mass transfer limitations. Similarly, according to Fick's Law, a decrease in the bubble size improves the mass transfer, resulting in faster CO_2 dissolution, slow rising, and high surface-to-volume ratio (AL-Mashhadani et al., 2015; Zimmerman et al., 2011).

- ***Mixing***

Mixing provides turbulence and homogeneity to the cultivation broth, preventing anaerobic conditions, light saturation and inhibition, and the formation of nutrient, gas, or thermal gradients (Eriksen, 2008; Ugwu et al., 2008). When algal growth is not limited by other parameters (such as light, nutrients or CO_2), efficient mixing is the key parameter determining the biomass yield as a result of its direct influence on light availability. In PBRs, agitation can be divided into mechanical (such as stirring, mechanical pumps, or the paddle wheels used in open ponds, where the shear rate is a function of the diameter of the impeller and the spinning rate) and non-mechanical through gas sparging in enclosed PBRs (Acién et al., 2017; Ugwu and Aoyagi, 2012).

Mixing optimization is crucial since a strong agitation could result in excessive shear stress, impairing algal metabolism and ultimately resulting in cell death, while low

mixing could lead to insufficient CO_2 mass transfer to the culture broth, limited oxygen removal, and poor access of microalgae to the photic zone in the PBR, thus limiting cell growth. Moreover, mixing is an important contributor to the energy consumption and therefore, to the operating costs of the process. In this sense, some authors have studied the potential reduction of energy consumption during mixing by modifying the reactor configuration. For instance, Zeng et al., (2016) assessed the performance of 15° inclined blades in a raceway pond, observing a better mixing for the same power consumption and an increase in biomass areal productivity of 15 %, while Zhang et al., (2013) studied the efficiency of a tubular PBR with helical static mixers, obtaining a biomass productivity 37.3 % higher than in conventional tubular PBRs.

Whereas linear velocities of 10 – 30 cm s^{-1} are commonly reported in HRAPs, this value depends on several factors such as the microalgae strain or the PBRs scale and configuration (Sullivan et al., 2003; Ugwu and Aoyagi, 2012). On the other hand, linear velocities of up to 1 m s^{-1} are recommended to prevent biofouling in tubular PBRs treating wastewater.

Technical and economic feasibility of photosynthetic biogas upgrading

Photosynthetic biogas upgrading has been proven as an innovative technology capable of producing a biomethane that complies with most standards for injection into the natural grid or use as a vehicle fuel. When compared with conventional physical-chemical biogas upgrading technologies currently available at commercial scale, which exhibit energy consumptions between 0.2 and 1 kWh per m^3 of biogas treated (Sun et al., 2015), a considerably lower energy demand of 0.08 kWh per m^3 of biogas treated is required when the biogas is photosynthetically upgraded in a HRAP interconnected to an external SC (Toledo-Cervantes et al., 2017a). This represents between ×2.5 and ×12.5 less energy consumed. On the contrary, photosynthetic biogas upgrading entails ~1.5 – 2.2 times higher investment costs and a larger footprint (Ángeles et al., 2019).

Interestingly, wastewater treatment in HRAPs through microalgae-bacteria symbiosis can reduce treatment costs (from 0.2 to 0.15 € m^{-3}) and energy consumption (up to 400 %) compared to conventional activated sludge processes (Acién et al., 2017). Algal biomass production from photosynthetic biogas

upgrading could boost the economic viability of this technology, yielding revenues from year 5 of operation when selling algal biofertilizers at 0.08 € kg^{-1} (considering a biomass productivity of 20 g m^{-2} d^{-1}) and biomethane at natural gas prices without incentives (Toledo-Cervantes et al., 2017a). Moreover, the produced microalgal biomass can reach sales prices up to 1.65 € (kg biomass)$^{-1}$ when devoted to the production of value added products such as lipids (used as feedstock for the chemical industry or transport fuel), proteins or polysaccharides (Da Silva et al., 2014) (see section 1.3). Nevertheless, a further evaluation of the application possibilities and the purity of microalgal biomass is necessary prior determination of their cost-benefit.

1.3 Algal biomass valorization into value added products

Microalgae are mainly composed of proteins (40 – 60 %), lipids (5 – 20 %), carbohydrates (20 – 30 %), and ashes (5 – 15 %) (Alcántara et al., 2015; Chisti, 2007). Several authors have assessed the potential of microalgae cultivation in a wide range of wastewaters (such as urban wastewater, swine waste, dairy manure, and poultry waste) combined with the simultaneous utilization of the resulting biomass for the production of different value added products such as biofuels, lipids, carbohydrates, proteins, vitamins, pigments, and biopolymers (Fig. 1.11) (Bhati and Mallick, 2016; Borowitzka and Moheimani, 2013; Hu et al., 2008; Olguín et al., 2003; Samantaray and Mallick, 2012). The investigation on microalgae-based wastewater treatment conducted in the past decade has focused on decreasing the HRT (to decrease the inherent high footprint of this process) and on upscaling high-rate algae ponds (to obtain precise data on energy consumption and validate the technology). Nevertheless, algal-bacterial photobioreactors present limitations such as poor process performance in carbon-limited wastewaters, poor biomass settling, limited number of process configurations and metabolic functions investigated, and limited understanding of the microalgae-based bioconversion processes into value added bioproducts in the context of wastewater treatment (Ángeles et al., 2019).
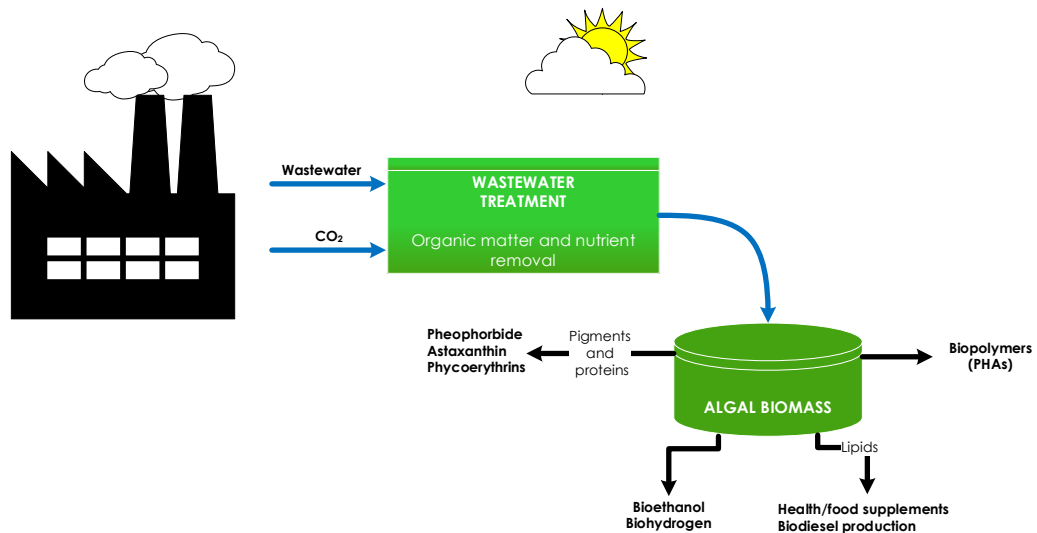

Fig. 1.11. Schematic representation of the potential valorization strategies of algal biomass into biofuels and value added products. Adapted from Haddadi et al., (2018).

Microalgae as feedstock for biofuel production

The production of third-generation biofuels such as biodiesel, bioethanol, biogas, or biohydrogen usually raises potential conflicts with food production. In this context, the use of microalgae as feedstock and their cultivation coupled with wastewater treatment has been widely explored as a cost-efficient and sustainable alternative (Collotta et al., 2018; Milano et al., 2016).

- ### *Biodiesel*

Microalgae are able to accumulate from 20 up to 70 % of lipids (on a dry matter basis) within their cells depending on the microalgae species and cultivation conditions (Chisti, 2007). This makes them attractive for biodiesel production as their fatty acid composition is similar to that of vegetable oil and animal fat. Lipid productivity and composition depend on several factors such as the liquid media composition (especially the nitrogen forms and concentrations), temperature, pH, CO_2 supplementation, or the cultivation mode (Koutra et al., 2018). For instance, Chisti, (2007) obtained a 77 % lipid content in *Schizochytrium* sp. and Illman et al. (Illman et al., 2000) achieved a 63 % lipid content in *C. emersonii* cultivated in low nitrogen medium. Zhu et al., (2014) cultivated *C. zofingiensis* in pilot scale PBRs with artificial wastewater in winter conditions, recording a lipid content > 50 % dry cell weight (dcw) suitable for good quality biodiesel production. Unfortunately, scarce research has been performed so far on tailoring the fatty acid profile, which is of key interest since it determines the biodiesel quality and properties (Koutra et al., 2018). Moreover, the cost competitiveness of algal-biofuel production over petroleum-based fuels is strongly dependent on algal-biomass yield and process improvements (Borowitzka and Moheimani, 2013; Sun et al., 2011).

- ### *Biogas and biohydrogen*

Green gaseous biofuels such as biogas can be obtained from the anaerobic digestion of algal biomass, which presents a competitive advantage over other biofuels since it does not require oil or lipids extraction and all the macromolecules of the microalgae are utilized during the methanogenic fermentation. Besides the typical factors affecting anaerobic digestion (organic loading and HRT,

temperature, pH, C/N ratio, inoculum to substrate ratio), biogas production from algal biomass digestion must also consider other parameters such as the microalgal species, the biomass pretreatment, and the previous algal cultivation conditions, in order to ensure an acceptable biogas yield and composition (Jankowska et al., 2017; Koutra et al., 2018). For instance, a low C/N ratio in the cultivation media of microalgae devoted to biogas production via anaerobic digestion may negatively impact the digestion process due to ammonia accumulation and pH increase (Koutra et al., 2018).

Biohydrogen production by cyanobacteria and green algae has emerged as a promising alternative to the present carbon-based fuels. In this sense, microalgae and cyanobacteria can produce biohydrogen by bio-photolysis (using light energy and converting water to H_2), or they can be used as a substrate for dark fermentation by anaerobic bacteria (Argun et al., 2017; Ferreira et al., 2012). Moreover, dark fermentation might be also combined with urban wastewater treatment using microalgae and the subsequent energetic valorization of the biomass obtained. Nevertheless, whereas the potential of biohydrogen production from microalgal biomass has been widely demonstrated, its production must be combined with other types of biofuels to ensure economic feasibility (Batista et al., 2015).

- *Bioethanol*

The cost-effective production of bioethanol from microalgal biomass relies on the adequate selection of the species and the cultivation substrate since their content in fermentable carbohydrates is usually relatively low for large-scale production (5 – 23 %). On the contrary, microalgae present highly fermentable carbohydrates and lack of lignin in comparison with terrestrial feedstocks (de Farias Silva and Bertucco, 2016; Koutra et al., 2018; Singh and Dhar, 2011). Despite the potential of ethanol production from microalgal biomass, there are still important constraints for its industrial implementation which makes it not economically favorable compared with current fossil fuel production. In this sense, research must focus on improving both the carbohydrate content and the biomass productivity, together with the optimization of hydrolysis and fermentation technologies (de Farias Silva and Bertucco, 2016).

Microalgal synthesis of value added products

The economic feasibility of large-scale production of biofuels from microalgae is usually constrained by the high capital and investment costs. Thus, the coproduction of value added products such as pigments, proteins, antioxidants, or biopolymers has been recently explored in order to increase the profitability of the process. In this sense, the high content in proteins found in microalgae cultivated in wastewater supports their application as biofertilizers (Mulbry et al., 2005; Sepúlveda et al., 2015). Microalgae are also capable of storing high concentrations of proteins such as astaxanthin, used as antioxidant agent, and phycobilin, employed as fluorescent labeling reagents. In addition, pigments such as pheophorbide A (utilized as photosensitizer in photodynamic therapy) are obtained from the breakdown of the chlorophyll synthesized by microalgae such as *Chlorella* sp. and *Spirulina* (Busch et al., 2009; Yen et al., 2013). Similarly, *D. salina* was the first microalgae used for the commercial production of β-carotene, frequently used in the pharmaceutical industry as a powerful antioxidant for human health. Previous studies demonstrated that *D. salina* was able to accumulate up to 14 % of β-carotene on a dcw basis (Borowitzka, 2005).

The synthesis of these high value added products is carried out through the CBB-cycle, where G3P is produced (Fig. 1.12). Then, G3P is available for the synthesis of pyruvate via glycolytic pathway, which can be used as the precursor for the synthesis of amino acids, lipids, pigments, and proteins (Liang et al., 2019). Some species of cyanobacteria can also synthesize glycogen (GL) and polyhydroxybutyrate (PHB) from 3-PG via the CBB cycle, as shown in Figure 1.13. PHB is produced from acetoacetyl-CoA by the enzyme β-ketothiolase, which is then reduced to hydroxybutyryl-CoA utilizing NADPH as the electron donor. Finally, D-3-hydroxybutyryl-CoA is polymerized to PHB by a PHA synthase (Kamravamanesh et al., 2018).
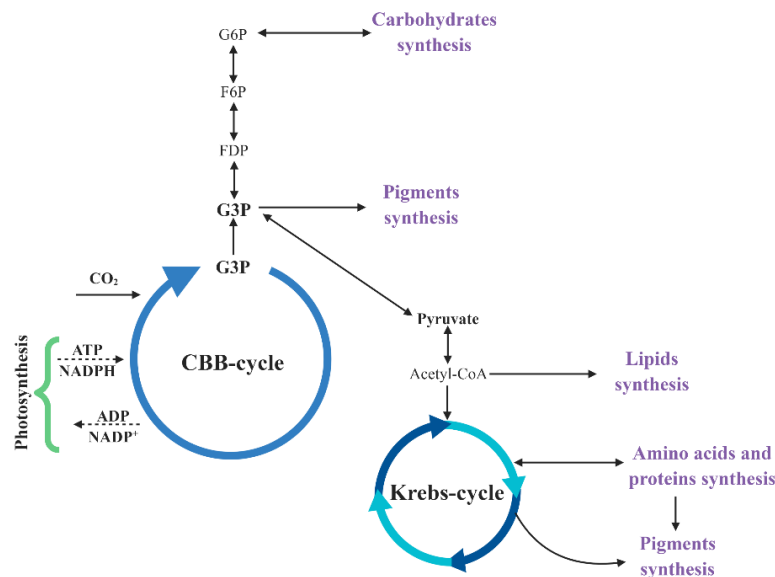
Fig. 1.12. Schematic diagram of the metabolic pathways for assimilation of carbon and production of high value added products in microalgae. Abbreviations: ADP, adenosine-diphosphate; ATP, adenosine-triphosphate; F6P, fructose-6 phosphate; FDP, fructose 1,6-biphosphate; G6P, glucose-6-phosphate; NADP+, nicotinamide adenine dinucleotide phosphate (oxidised); NADPH, nicotin-amide adenine dinucleotide phosphate (reduced); G3P, glyceraldehyde–3–phosphate. (Source: Adapted from Perez-Garcia et al., (2011). Figure created with BioRender.com).
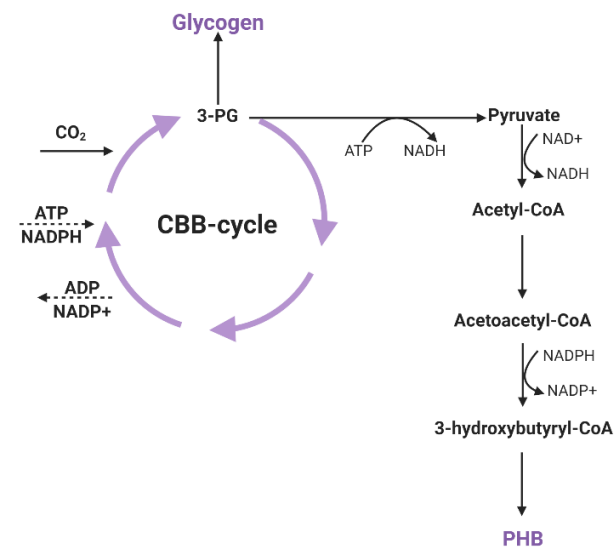

Fig. 1.13. Simplified biosynthetic pathways for polyhydroxybutyrate (PHB) and glycogen (GL) production in cyanobacteria. Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; NADP+, nicotinamide adenine dinucleotide phosphate (oxidised); NADPH, nicotin-amide adenine dinucleotide phosphate (reduced); NAD+, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced). (Source: Adapted from Kamravamanesh et al., (2018). Figure created with BioRender.com).

- *Polyhydroxyalkanoates*

Polyhydroxyalkanoates (PHAs) (Fig. 1.14 a), polymers produced by living organisms, have recently emerged as a sustainable alternative to conventional plastics obtained from non-renewable fossil fuels. They are produced from natural substrates under unbalanced nutrient conditions (i.e., N limitation) and are completely biodegradable and biocompatible, with thermal and mechanical properties similar to those of petroleum-derived plastics (such as polyethylene or polypropylene). By varying the producing strains, substrates, and co-substrates, a number of polyesters can be synthesized with different monomer composition. In particular, PHAs such as PHB (Fig. 1.14 b), poly-3-hydroxyvalerate (PHV), and their copolymer PHB-V, are polyesters with structural properties comparable to polypropylene, which are synthesized and stored intracellularly by bacteria, serving as energy and carbon storage compounds (Table 1.4) (Shrivastav et al., 2010).
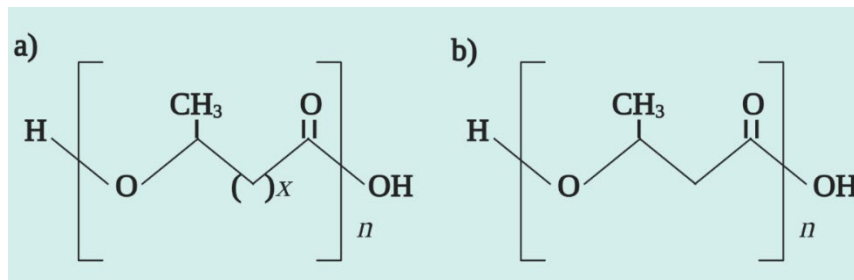

Fig. 1.14. Chemical structure of a) polyhydroxyalkanoates (PHAs) and b) polyhydroxybutyrate (PHB). (Figure created with BioRender.com).

PHAs have numerous applications in several sectors, including pharmaceutical and medical use, agriculture, biofuels production or packaging (López et al., 2018; Singh and Rathore, 2016). Depending on the monomer composition, the carbon source, the microbial strain used, and the purity of the final biocomposite, the market price varies from 4 up to 20 € kg_{PHA}^{-1}. This price is still not competitive compared to that of fossil-based polyesters, the high costs of carbon source acquisition accounting for 30 – 40 % of the final PHA price (López et al., 2018). In this sense, the accumulation of PHAs by microalgae and cyanobacteria cultivated in wastewater might improve the economic balance, boosting the competitiveness of biologically based polymers.

Table 1.4. Physical properties of PHB and the conventional polymer polypropylene (Balaji et al., 2013).

Properties	PHB	Polypropylene
Melting temperature (°C)	177	176
Glass transition temperature (°C)	2	−10
Crystallinity (%)	60	50 − 70
Tensile strength (MPa)	43	38
Extension to break (%)	5	400

Several studies have evidenced the capacity of cyanobacteria to accumulate intracellular PHAs under nutrient deprivation conditions (Table 1.5). For instance, *Arthrospira subsalsa* was able to accumulate 14.7 % dcw of PHB under N limitation (Shrivastav et al., 2010); *Synechococcus* sp. MA19 and *Nostoc muscorum* exhibited intracellular PHB contents of 55 and 21.5 % dcw, respectively, when cultivated under P limitation and CO_2 addition (Haase et al., 2012; Nishioka et al., 2001), while a 13 % dcw of intracellular PHB was achieved in *Synechocystis* sp. PCC 6714 under N and P limitation (Kamravamanesh et al., 2017). Some strains also require the supplementation with an organic carbon substrate to promote biopolymer production. For example, *Aulosira fertilissima* accumulated PHA up to 77.2 % dcw and 58.6 % dcw under P and N deficiency, respectively, with 0.5 % acetate supplementation (Samantaray and Mallick, 2015).

All these experiments were performed using a synthetic cultivation medium, while low-cost substrates for microalgae growth and PHB accumulation are preferred from an economic point of view. In this sense, the combination of wastewater or digestate treatment with biopolymers synthesis has been also successfully achieved for some cyanobacteria species. As an example, Bhati and Mallick, (2016) demonstrated the capacity of *N. muscorum* to accumulate up to 65 % of [P(3HB−co−)3 HV] when cultivated in poultry waste and supplemented with a 10 % CO_2-laden air stream. Arias et al., (2018) optimized the light/dark cycle and nutritional regime to enhance the intracellular accumulation of PHB and carbohydrates by a mixed wastewater-borne cyanobacterial culture, reaching 5.7 % dcw accumulation under P limitation and constant illumination. Moreover, natural digestate was evaluated as a nutrient solution for producing PHB by *Synechocystis salina* in a 200 L tubular PBR. While a maximum PHB content of ~ 6 % was obtained, the quality of the produced PHB in terms of thermal and rheological properties was similar to commercial PHB (Meixner et al., 2016; Troschl et al., 2018).

In brief, preliminary studies have demonstrated that cyanobacteria are able to achieve comparable PHB yields to bacterial strains, thus representing a potential alternative for PHA production. Besides, they allow for a reduction of the costs associated to biopolymer production using wastewater as a low-cost nutrient source. However, downstream processing is more difficult due to the lower cell densities of cyanobacterial cultures, which constitutes the main drawback for full-scale implementation.

- *Glycogen*

Polysaccharides are complex biochemical structures based on glycosidically-linked combinations of up to 40 - 50 different monosaccharides such as hexoses and pentoses (Delattre et al., 2016). Cellulose, starch, and GL are the most common polysaccharides in plants and photosynthetic microorganisms (Fig. 1.15).
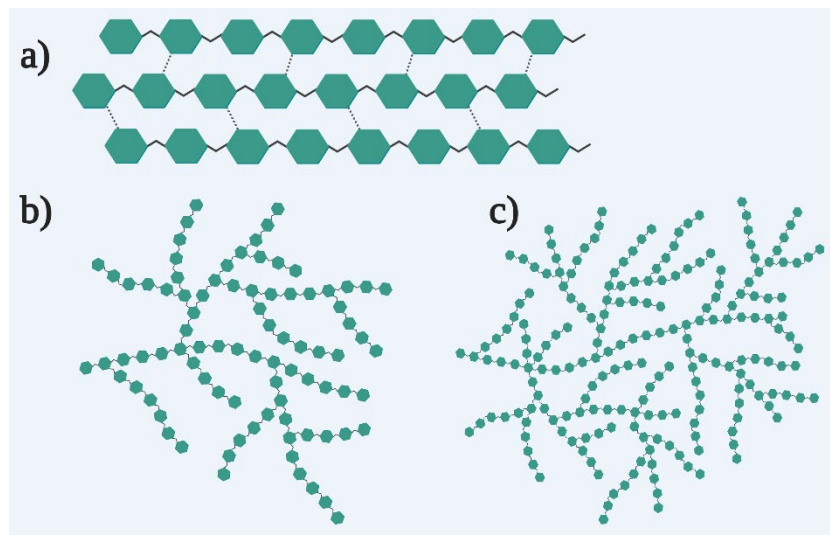

Fig. 1.15 Molecular structure of essential polysaccharides: a) cellulose, b) starch, and c) glycogen. (Figure created with BioRender.com).

Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage for cyanobacteria under nitrogen deprivation conditions. GL is synthesized in the illuminated period of the photosynthesis according to light availability, and involves the key enzyme ADP-glucose pyrophosphorylase (AGPase) (Kamravamanesh et al., 2019). Due to its unique properties such as high bio-compatibility and bio-degradability, high availability, and high water solubility,

GL is commonly applied in the pharmaceutical industry as a drug delivery vehicle (Gopinath et al., 2018). Additionally, GL can be used as a low-cost carbohydrate source for bioethanol production through saccharification and fermentation processes (Alam and Wang, 2019; Klein et al., 2015).

For instance, Aikawa et al., (2014) reported accumulations of up to 65 % dcw of GL by the euryhaline cyanobacteria *Synechococcus sp.* strain PCC 7002 obtained from an oceanic environment. Similarly, *Synechocystis* sp. PCC 6714 reached GL concentrations of ~76 % dcw using phosphate feeding, carbon dioxide as carbon source, and no N source (Kamravamanesh et al., 2019). These authors also investigated the potential for simultaneous accumulation of GL and biopolymers under the tested conditions, although PHB contents lower than 20 % dcw were always obtained. At this point, it is important to highlight that the GL synthesis pathway competes with that of PHB at the level of the 3–phosphoglycerate (3–PG) pool (Fig. 1.13) (Singh et al., 2017). To overcome this limitation, the inactivation of the pathway for GL synthesis in cyanobacteria through genetic engineering has been investigated. As reported by Khetkorn et al., (2016), a genetically modified *Synechocystis* sp. PCC 680 reached contents of up to 35 % dcw of PHB. Despite the application of genetic and metabolic engineering may enhance the conversion of CO_2 into value added products in cyanobacteria (such as ethanol, hydrogen, glycerol, isobutanol, lactic acid, or PHB), it is still necessary to determine the biomass yields at an industrial scale and the financial viability of the process (Carpine et al., 2017).

Table 1.5. Accumulation of biopolymers in cyanobacteria under different cultivation conditions.

Cyanobacteria species	Culture condition			Type of polymer	mg L^{-1}	(% dcw)	Reference
	Cultivated	Nutrient deficiency (N or P)	Supplementation				
Synechoccus sp MA19	BG-11	P	50 °C	PHB	-	62	Nishioka et al., (2001)
N. muscorum	BG-11	-	0.2 % acetate Dark incubation	PHB	-	35	Sharma and Mallick, (2005)
Chrococcus sp. M1	BG-11 modified Allen's	-	-	PHB	140	63.57	Beyatli et al., (2005)
Osillatoria limosa 1966/1380		-	-		55	85.45	
Chrococcus sp. M1	BG-11	-	-	PHB	145	18.62	
Osillatoria limosa 1966/1380		-	-		705	10.64	
Synechocytis sp. PCC6803	BG-11	P	0.4 % (w/v) acetate Pre-grown in BG-11 + 0.1 % glucose	PHB	-	28.8	Panda et al., (2006)
		N			-	14.4	
N. muscorum	BG-11	N	0.2 % acetate 0.4 % propionate	[P(3HB–co–)3HV]	-	28.2	Mallick et al., (2007a)
Arthrospira subsalsa	Modified ASNIII	N	5 % NaCl	PHA	147.75*	7.45	Shrivastav et al., (2010)
Aulorisa fertiluissima	BG-11	P	0.5 % (w/v) acetate	PHB	160.1	77.2	Samantaray and Mallick, (2012)
		N			143.5	58.6	
N. muscorum	ES medium	P	Aeration 2.4L h^{-1} air + 1.8 mL d^{-1} CO$_2$	PHB	-	21.5	Haase et al., (2012)
N. muscorum	BG-11	N	10 % Poultry litter 10 % CO$_2$ air flow 0.28 % acetate 0.38 % glucose 0.30 % valerate	PHA	773.5	65	Bhati and Mallick, (2016)
S. salina	Digestate supernatant	-	-	PHB	88.7	5.5	Meixner et al., (2016)
Synechocytis sp. PCC 6714	BG-11	N and P	2 % CO$_2$ at a flow rate of 0.02 vvm (20 mL min^{-1})	PHB	342	14.6	Kamravamanesh et al., (2017)
Mixed cyanobacterial culture	Wastewater	N	12:12 cycles light: dark	PHB	61.61	6.5	Arias et al., (2018)
		P	24:0 cycles light: dark		104	5.7	

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Chapter 2

Aims and Scope of the Thesis

2.1 Justification of the Thesis

The development of renewable energies such as biomethane, which is produced via biogas upgrading, provides a promising alternative for the reduction of CO_2 and other greenhouse gas emissions. The large-scale production of biomethane will contribute to the European political initiatives for climate change mitigation and the promotion of renewable energies. However, the biogas upgrading market is nowadays dominated by physical-chemical technologies, which limits the widespread use of biomethane as a renewable energy source due to their high energy demand, operating costs and chemical requirements. In this context, photosynthetic biogas upgrading using microalgae could be a potential technology to support a cost-efficient and environmentally-friendly removal of undesired components from biogas (i.e., CO_2 and H_2S) in one stage, with the concomitant production of valuable algal biomass. However, the performance of photosynthetic biogas upgrading is still limited by the high O_2 and N_2 desorption from the cultivation broth to the biomethane, and by the limited know-how of this process when implemented in enclosed PBR and the need to operate in the presence of light to fix CO_2 and oxidize H_2S. The uptake of this green technology by the industrial sector requires the engineering of innovative design and operational strategies to i) prevent O_2 and N_2 contamination of biomethane, ii) to fully exploit the advantages of enclosed PBR, and iii) to upgrade biogas even in the dark period. Moreover, algal biomass valorization into value added products is crucial to compensate for the high investment costs of photosynthetic biogas upgrading and to recover the nutrients contained in the wastewater. Hence, the use of microalgae-based processes increases the environmental and economic benefits of biogas upgrading and contributes to the development of circular economy models.

2.2 Main objectives

The main objective of this PhD thesis was the engineering of innovative operational strategies to obtain a biomethane composition complying with most international standards for utilization as a vehicle fuel or injection into natural gas grids under two different photobioreactor configurations. This approach combined a reduction in

the costs of biogas upgrading using photosynthetic processes with the creation of a circular economy model through biomass valorization. More specifically:

Objective 1- to explore the potential of innovative operational strategies to minimize the content of N_2 and O_2 in the upgraded biogas in open PBRs.

Objective 2- to evaluate the influence of the alkalinity of the cultivation broth on the biogas upgrading performance of an enclosed tubular-PBR interconnected to an external biogas SC.

Objective 3- to investigate the influence of nitrogen deprivation cycles and biogas supply regime on the photosynthetic biogas upgrading performance of the tubular-PBR interconnected to a biogas SC.

Objective 4- to evaluate the effect of nitrogen deprivation cycles and biogas supply regime on the production of algal biomass and its chemical composition.

Objective 5- to optimize both the growth of the cyanobacterium *N. muscorum* and the accumulation of GL and PHB using CO_2 from biogas as the carbon source.

2.3 Development of the Thesis

In the present thesis, the performance of photosynthetic biogas upgrading combined with algal biomass valorization was evaluated under different operational strategies and photobioreactor configurations.

Thus, two new operational strategies aiming at minimizing the content of N_2 and O_2 in the upgraded biogas in a conventional open PBR were explored in *Chapter 3*. The first approach was based on the degassing of the culture broth using PDMS liquid-gas membranes operated at varying vacuum pressures before being fed to the SC. The second approach focused on minimizing the desorption of N_2 and O_2 from the liquid to the biomethane using an external pressurized SC. In *Chapter 4*, an enclosed tubular-PBR was used to evaluate the influence of the alkalinity of the cultivation broth on biomethane quality. In addition, after optimization of the culture conditions for integral biogas upgrading, the tubular-PBR was operated under nitrogen deprivation cycles with the purpose of assessing the influence on the chemical composition of the algal biomass (*Chapter 4*). Furthermore, the influence of the biogas supply regime (feeding only during the illuminated period or continuous feeding during the dark/light periods) on the photosynthetic biogas

upgrading performance and algal biomass production was investigated in *Chapter 5.*

Finally, *Chapter 6* focused on the optimization of the growth of the cyanobacterium *N. muscorum* and the accumulation of value added products such as GL and PHB using CO_2 from either biogas or flue-gas as the carbon source under different nutrient deprivation strategies.

Chapter 3

Strategies for N$_2$ and O$_2$ removal during biogas upgrading in a pilot algal-bacterial photobioreactor

Strategies for N_2 and O_2 removal during biogas upgrading in a pilot algal-bacterial photobioreactor

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Highlights

- Biomethane from photosynthetic biogas upgrading usually contains N_2 and O_2.
- Membrane degasification of the cultivation broth reduced N_2 and O_2 concentrations.
- Biogas scrubbing at 2 atm achieved high CO_2 and H_2S removals (~98 and 100 %).
- Both strategies provided a high quality biomethane in terms of N_2 and O_2 content.

Abstract

Photosynthetic biogas upgrading in algal-bacterial photobioreactors interconnected to external scrubbing columns (SCs) has been proven as a feasible, low cost and environmentally friendly technology, ensuring a high removal performance of the main biogas contaminants (i.e., CO_2 and H_2S). However, despite the promising results, the upgraded biogas is commonly contaminated with N_2 and O_2 desorbed from the cultivation broth, which limits biomethane applications. In this work, two innovative strategies to improve the quality of biomethane were evaluated: degasification of the cultivation broth by means of a polydimethylsiloxane (PDMS) gas-liquid membrane, and biogas scrubbing at high pressure. The hollow fiber PDMS membrane demonstrated a superior degassing performance at 100 − 300 mbar of vacuum pressure, exhibiting dissolved oxygen removals of 61.7 ± 7.8 % in the cultivation broth and reducing N_2 and O_2

concentrations in the upgraded biomethane to ~0.8 and ~0.1 %, respectively. On the other hand, biogas scrubbing at 2 atm (over atmospheric pressure) promoted CO_2 and H_2S mass transfer from the biogas to the recirculating broth, achieving removals of ~98 and 100 %, respectively, and N_2 and O_2 concentrations in the biomethane of ~1.7 and ~0.1 %, respectively. Both strategies provided a biomethane composition complying with most international standards for utilization as vehicle fuel or injection into natural gas grids.

Keywords: *Biogas Upgrading, Biomethane, Microalgae, PDMS Membrane, Pressurized Scrubbing Column*

3.1   Introduction

Biogas from the anaerobic degradation of organic matter is a sustainable vector to obtain renewable energy (Muñoz et al., 2015; Sahota et al., 2018). Raw biogas is composed of CH_4 (40 − 75 %), CO_2 (15 − 0 %), H_2S (0.005 − 2 %), O_2 (0 − 2 %), N_2 (0 − 3 %), NH_3 (< 1 %), CO (< 0.6 %), VOC (< 0.6 %), water (5 − 10 %) and siloxanes (0 − 0.02 %) (Ryckebosch et al., 2011). The removal of biogas contaminants is of key importance to ensure a cost-effective use of this energy vector, either as vehicle fuel or for injection into natural gas grids (with current technical specifications of CH_4 > 90 − 95 %, CO_2 < 2 %, O_2 ≤ 0.001 −1 %, N_2 < 2 − 10 % and H_2S + COS < 5 mg Nm^{-3}) (Jouzani, 2018; Sun et al., 2015). While some contaminants such as CO_2 and N_2 decrease the calorific value of biogas, others such as H_2S are toxic and corrosive, significantly reducing the lifespan of biogas storage structures, pipelines, boilers and internal combustion engines (Angelidaki et al., 2018; Toledo-Cervantes et al., 2017a). On the other hand, O_2 can entail explosion risk. In this context, physical-chemical biogas upgrading technologies have been developed and commercialized at industrial scale, and represent the market-leading technologies for biogas upgrading nowadays (EBA, 2018). However, their high energy consumption, operating cost and chemical requirements, together with the complex sequences required for integral biogas upgrading, still limit the environmental and economic sustainability of these technologies (Muñoz et al., 2015).

Photosynthetic biogas upgrading in algal-bacterial photobioreactors constitutes a sustainable and environmentally friendly alternative for the simultaneous removal of CO_2 and H_2S from biogas. This technology is based on CO_2 fixation by microalgae via photosynthesis and the concomitant H_2S oxidation by sulphur-oxidizing bacteria utilizing the oxygen photosynthetically produced (Bahr et al., 2014). The potential of this innovative technology has been demonstrated both indoors and outdoors in pilot and demo high rate algal ponds (HRAP) interconnected to biogas scrubbing column (SC) (Bahr et al., 2014; Posadas et al., 2015a; Rodero et al., 2018a; Serejo et al., 2015; Toledo-Cervantes et al., 2017b), achieving removal efficiencies > 98 % for CO_2 and an almost complete H_2S abatement. This strategy was able to guarantee a biomethane quality with concentrations of CH_4 of > 87 %, CO_2 of 0.9 − 9 %, N_2 of 0.7−9 % and O_2 of 0 − 2 %. Despite the satisfactory results obtained for CO_2 and H_2S, previous experiments did not result in a biomethane composition complying with most biomethane standards due to the contamination of the upgraded biogas with N_2 and O_2 (stripped out from the cultivation broth in the SC).

Therefore, two new operational strategies to minimize the content of N_2 and O_2 in the upgraded biogas were investigated in this study. The first approach aimed at reducing the content of both gases in the

biomethane via degassing of the culture broth before being fed to the SC. For this purpose, the use of polydimethylsiloxane (PDMS) liquid-gas membranes operated at varying vacuum pressure was assessed. The second approach evaluated the potential of an external pressurized SC to minimize the desorption of N_2 and O_2 from the liquid to the biomethane. To the best of the authors knowledge, these strategies have never been implemented before in a HRAP-SC system for integral biogas upgrading.

3.2 Materials and methods

3.2.1 Mineral salt medium

The mineral salt medium (MSM) was composed according to Rodero et al., (Rodero et al., 2019) Inorganic carbon (IC) was added at a concentration of 1500 mg L^{-1} using a mixture of $NaHCO_3$, 7.5 g L^{-1} and Na_2CO_3, 3.7 g L^{-1} (Rodero et al., 2018b). All chemicals were purchased from Panreac® (Barcelona, Spain) with a purity of at least 99 %.

3.2.2 Experimental set-up

The experimental set-up consisted of a HRAP of 15 cm depth, 202 cm length and 63 cm width, with a working volume of 180 L and an illuminated area of 1.2 m^2 at 1378 ± 166 µmol m^{-2} s^{-1} by six high intensity LED PCBs (Phillips SA, Spain) using 12:12 h light:dark periods (Fig. 3.1). The HRAP was continuously agitated by a six-blade

paddlewheel at an internal cultivation broth recirculation velocity of 20 cm s^{-1}. The HRAP and SC (2.5 L, 165 cm height, 4.4 cm internal diameter) were interconnected via external recirculation of the biomass-free cultivation broth from an 8 L settler. The AC was fed during the illuminated period by means of a stainless-steel diffuser (2 µm pore size, located at the bottom of the column) with a synthetic biogas mixture (70 % of CH_4, 29.5 % of CO_2 and 0.5 % of H_2S, Abello Linde (Spain)). The synthetic biogas was fed at a gas flowrate G = 39.4 ± 2.2 L d^{-1}, while the cultivation broth recirculation was maintained at a flowrate of L = 20.1 ± 0.2 L d^{-1}, resulting in a L/G ratio of ~0.5. In order to improve the removal of total suspended solids from the settler supernatant, a polyacrylamide-based flocculant solution (Chemifloc CV-300) was dosed at 240 mg L^{-1} to recover the algal-bacterial biomass by coagulation-flocculation. Biomass was daily harvested from the flocculation tank in order to maintain a productivity of ~4 g m^{-2} d^{-1} (16.9 g d^{-1}). Distilled water was daily supplied to the HRAP to compensate water evaporation losses. The system was fed with MSM at a hydraulic retention time of 56.3 days.

3.2.3 Approach 1: Degassing of the cultivation broth using a gas-liquid membrane

3.2.3.1 Evaluation of the degassing capacity of flat-plate and hollow fiber membranes

A PDMS flat membrane (total surface of 150 cm^2, Pervatech, The Netherlands) and a hollow fiber PDMS membrane (PDMSXA-1000 module, 1280 fibers with an inner diameter of 0.3 mm, total surface of 1000 cm^2, PermSelect©) were in terms of their ability to remove dissolved oxygen (DO) from the cultivation broth in order to elucidate the most suitable membrane configuration. To this end, air-saturated MSM at an IC concentration of 1500 mg L^{-1} was supplied at different liquid flow rates (5, 10, 20, and 30 mL min^{-1}) through the membranes, and the gas side was connected to a vacuum pump (V-700V, Büchi, Switzerland) operated at 100 mbar. Two oximeters were placed at the inlet and outlet of the membrane and the DO concentration was monitored every five minutes until a steady state for the biomethane composition was reached (Fig. 3.1).

3.2.3.2 Continuous operation with the hollow fiber gas-liquid membrane and influence of the vacuum pressure on biogas upgrading performance

The photobioreactor was operated for 20 days prior membrane installation in order to determine the steady state photosynthetic biogas upgrading performance (Fig. S1).

Afterwards, the performance of the hollow fiber PDMS membrane, located in the recirculating stream between the settler and the SC, was evaluated. The influence of the membrane operation on the quality of the upgraded biogas was evaluated at ~150 mbar of vacuum pressure for 16 days. An additional test assessing the influence of the vacuum pressure (100, 300 and 900 mbar) on the membrane degassing capacity and on the quality of the upgraded biogas was performed. The DO concentration in the biomass-free cultivation broth at the inlet and outlet of the membrane was continuously monitored for 5 h per day for 5 days at each vacuum pressure. The DO concentration at the membrane outlet was allowed to stabilize before the analysis of the composition of the upgraded biogas.

3.2.4 Approach 2: Process operation in a pressurized biogas scrubbing column

The SC was operated with two metering valves (1315G4Y, Hoke, United States) to provide an accurate control of the outlet liquid and gas flowrates, and therefore of the pressure inside the column. The influence of the SC operating pressure (0, 1 and 2 atm) on biomethane composition was evaluated under steady state for at least 4 consecutive days at each operating pressure (Fig. S2).

3.2.5 Analytical procedures

CH$_4$, CO$_2$, H$_2$S, N$_2$, and O$_2$ gas concentrations were analyzed by gas chromatography in a Bruker 430 GC-TCD (Bruker, Palo Alto, USA) equipped with a CP-Molsieve 5A and a CP-PoraBOND Q columns. The oven, injector and detector temperatures were maintained at 45, 150 and 200 °C, respectively. Helium was used as the carrier gas at 13.7 mL min^{-1}.

The DO concentration was monitored in the cultivation broth of the HRAP, inlet and outlet of the gas-liquid membranes using the oximeters Oxi 3310 and Oxi 330i (WTW,

Germany). The pH was determined with a Eutech Cyberscan 510 pH meter (Eutech Instruments, The Netherlands), while dissolved IC, total organic carbon (TOC) and total nitrogen (TN) in the cultivation broth were determined using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. The determination of VSS concentration was carried out according to Standard Methods, and N-NO$_2^-$, N-NO$_3^-$, P-PO$_4^{3-}$ and S-SO$_4^{2-}$ concentrations were quantified by HPLC-IC according to Posadas et al., (2013).
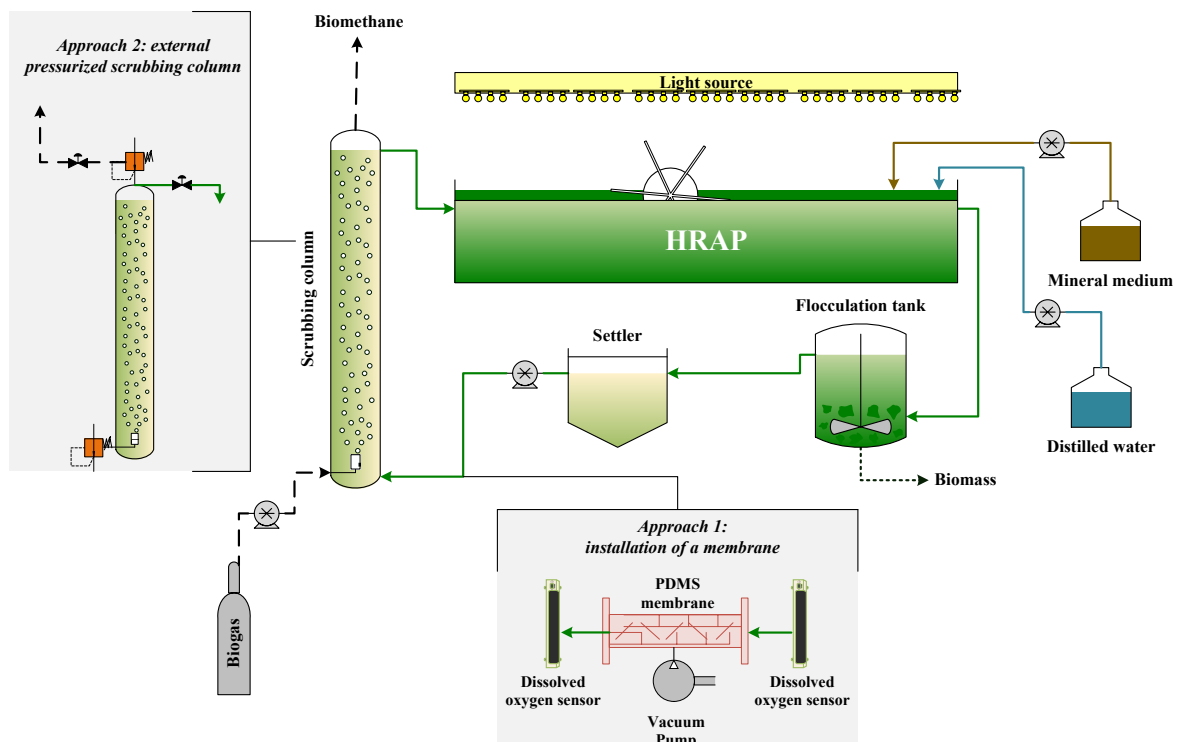
Fig. 3.1. Schematic representation of the experimental setup. Approach 1: installation of a gas-liquid membrane between the settler and the biogas scrubbing column. Approach 2: external pressurized scrubbing column.

3.3 Results and discussion

3.3.1 Approach 1: Degassing of the cultivation broth using a gas-liquid membrane

The flat PDMS membrane showed a poor degassing capacity even at the lowest liquid flowrate tested, with the value of 16.7 ± 2.2, 18.4 ± 3.6 and 35.5 ± 16.2 mg DO m^{-2} h^{-1}, corresponding to average DO removal efficiencies (REs) of 16.0 ± 1.8, 8.9 ± 1.7 and 7.7 ± 3.3 % at 5, 10, and 20 mL min^{-1}, respectively (Fig. 3.2a). This poor performance was attributed to the limited surface per unit volume of the flat membrane, while increasing the liquid flowrate resulted in a reduced residence time in the membrane module and the likely formation of preferential flow paths. On the contrary, the hollow fiber PDMS membrane supported DO-REs of 72.5 ± 2.7 and 60.30 ± 5.4 % at 5, and 10 mL min^{-1}, respectively, increasing to ~ 87 % at 30 mL min^{-1}. At this highest liquid flowrate, the hollow fiber PDMS membrane exhibited a specific degassing capacity of 92.2 ± 2.5 mg DO m^{-2} h^{-1}. These results confirmed the potential of the hollow fiber PDMS membrane to reduce the dissolved O_2 content in the recirculating cultivation broth and its superior performance over the flat membrane tested (Baker, 2004).

After selection of the most suitable membrane configuration, the system was operated for 20 days maintaining a high alkalinity in the cultivation broth of 1380 ± 55.7 mg IC L^{-1} without membrane. Average

CO_2 and H_2S-REs of 94.0 ± 0.6 and 93.8 ± 0.01 %, respectively, were recorded, resulting in a biomethane composition of 96.4 ± 0.3, 1.9 ± 0.2, 0.04 ± 0.01, 1.5 ± 0.2 and 0.2 ± 0.04 % for CH_4, CO_2, H_2S, N_2 and O_2,
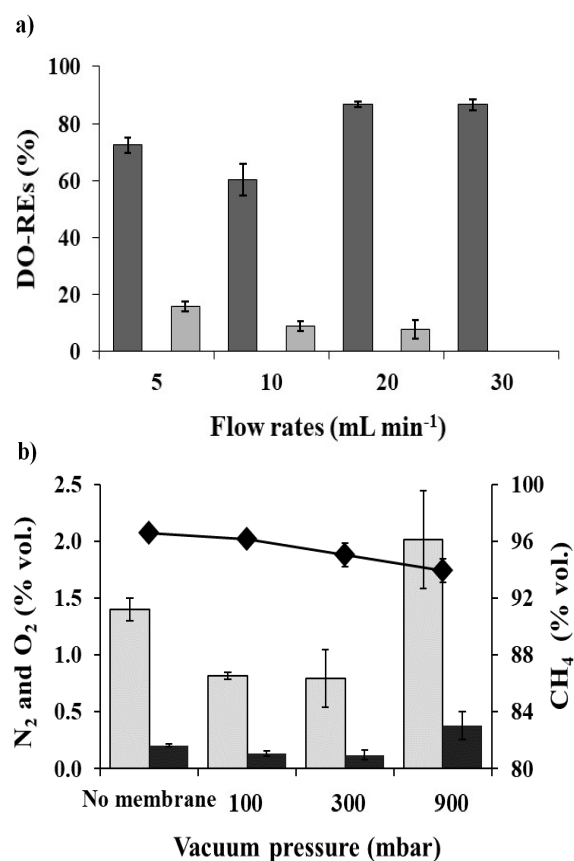

Fig. 3.2. a) Influence of the liquid flowrate on the average dissolved oxygen removal efficiencies (DO-REs) and their standard deviation (± SD, n=6 − 8) of the flat PDMS membrane (light grey bars) and the hollow fiber PDMS membrane (dark grey bars). b) Influence of the operating pressure in the absorption column on the average O_2 (black bars), N_2 (grey bars) and CH_4 (diamonds) concentration in the upgraded biogas and their standard deviation (± SD, n = 4). Vertical bars represent steady state standard deviations.

respectively (Fig. 3.2b). Under these operating conditions, the photosynthetic activity of microalgae resulted in a DO concentration of 15.0 ± 1.7 mg O_2 L^{-1} in the cultivation broth. It is important to remark that the quality of the upgraded biogas here obtained was noticeably higher than that reported in previous studies, where higher O_2 concentrations have been consistently reported (Bahr et al., 2014; Meier et al., 2017; Serejo et al., 2015). The hollow fiber PDMS membrane was then installed and operated under continuous mode at ~ 150 mbar of vacuum pressure for 16 days, further improving the quality of the biomethane in terms of O_2 and N_2 concentration (average biomethane composition of 96.3 ± 0.4, 2.5 ± 0.4, 0.03 ± 0.01, 1.0 ± 0.2 and 0.2 ± 0.04 % for CH_4, CO_2, H_2S, N_2 and O_2, respectively). A statistically significant (Anova test, $p > 0.05$) lower concentration was recorded for these biogas contaminants, with a reduction of 33 and 37 % in the concentration of O_2 and N_2 in the upgraded biogas, respectively.

Finally, the influence of the vacuum pressure (100, 300 and 900 mbar) on the membrane specific degassing capacity was evaluated. Although no statistically significant differences were observed in the concentration of CH_4 (~ 96 %), N_2 (~ 0.8 %) and O_2 (~ 0.1 %) when operating at 100 and 300 mbar of vacuum pressure, a reduced vacuum resulted in a substantial deterioration of the quality of the upgraded biomethane, with average concentrations of 93.9 ± 0.8 % for CH_4, 3.7 ± 0.3 % for CO2, 0.03 ± 0.0 % for H_2S, 2.0 ± 0.4

% for N_2 and 0.4 ± 0.1 % for O_2. Interestingly, the DO-REs in the cultivation broth recorded during the operation with the hollow fiber PDMS membrane decreased from 61.7 ± 7.8 % at 100 mbar of vacuum pressure to 3.5 ± 16.0 and -26.3 ± 31.2 % at 300 and 900 mbar, respectively, which corresponded to a reduction in the specific degassing capacity from 26.2 ± 5.7 to 2.1 ± 6.0 and -8.0 ± 9.6 mg DO m^{-2} h^{-1}, respectively. In this context, Meier et al., (Meier et al., 2017) estimated that, under atmospheric pressure conditions, 5% of the photosynthetic O_2 produced in the
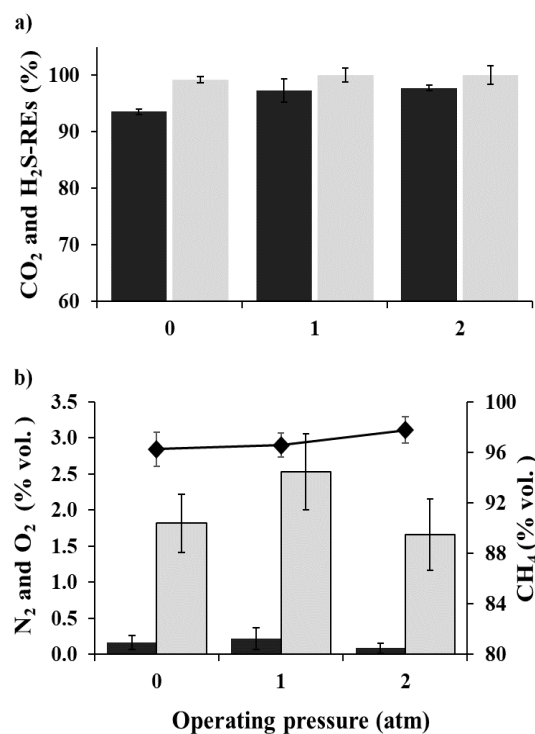

Fig. 3.3. a) Removal efficiency (REs) of CO_2 (black bars) and H_2S (grey bars) at different overpressures. b) Average O_2 (black bars), N_2 (grey bars) and CH_4 (diamonds) concentration in the upgraded biomethane at different overpressures. Vertical bars represent steady state standard deviations ($\pm$ SD, n = 6 – 12).

photobioreactor was transferred to the upgraded biomethane, the rest being desorbed from the cultivation broth.

3.3.2 Approach 2: Process operation in a pressurized biogas absorption column

CO_2 and H_2S-REs of 93.5 ± 2.1 and 99.2 ± 1.0 % were obtained when the SC was operated under atmospheric pressure (Fig. 3.3a), which resulted in a biomethane composition of 96.3 ± 1.4, 2.3 ± 0.8, 0.0 ± 0.0, 1.8 ± 0.4, and 0.2 ± 0.1 % for CH_4, CO_2, H_2S, N_2 and O_2, respectively. Process operation under pressure boosted the mass transfer of CO_2 and H_2S from the biogas to the liquid phase, achieving REs of 97.3 ± 0.8 and 97.8 ± 1.9 % for CO_2 at 1 and 2 atm, respectively, and H_2S concentrations below the detection limit of the GC-TCD (~ 0.17 g m^{-3}). Consequently, CH_4 concentration increased from ~ 96 % at 0 and 1 atm up to 97.8 ± 1.0 % when operating at overpressures of 2 atm (Fig. 3.3b).

Nevertheless, despite the satisfactory results obtained for CO_2 and H_2S removal, O_2 and N_2 desorption from the cultivation broth was identified as the main drawback of this technology when working at 1 atm of overpressure. It was hypothesized that a higher pressure entailed a reduction in the bubble size and, therefore, an increase in the specific area and the mass transfer coefficient (k_La) from the liquid to the gas phase, which could not compensate the higher aqueous solubility of O_2 and N_2 at

higher pressures (Serejo et al., 2015). Interestingly, a further increase in the pressure of the SC up to 2 atm was able to overcome this physical effect and sufficiently increase the aqueous solubility of O_2 and N_2 to prevent their desorption from the cultivation broth. In this context, N_2 concentrations in the upgraded biomethane accounted for 1.8 ± 0.4, 2.5 ± 0.5 and 1.7 ± 0.5 % at 0, 1 and 2 atm, respectively, with O_2 concentrations of 0.2 ± 0.1, 0.2 ± 0.2 and 0.1 ± 0.1 % (Fig. 3.3b).

3.3.3 Technical feasibility analysis of the proposed strategies

Further research and optimization of both strategies at laboratory and pilot scale is necessary prior determination of their cost-benefit ratio. Moreover, the techno-economic feasibility of implementing these strategies for integral biogas upgrading must be carefully analyzed on a case-by-case basis. According to a previous analysis conducted by the authors Toledo-Cervantes et al. (Toledo-Cervantes et al., 2017a), power consumption in a HRAP-SC system treating 300 Nm^3 h^{-1} of raw biogas accounts for ~208000 kW-h y^{-1}, compression costs representing only ~8 % of the total energy needs. However, the operation of the absorption column under pressure would significantly increase the energy requirements. In this context, an overpressure of 2 bar will result in a ×13.4 increase in the compression power consumption. Nevertheless, it is important

to remark that these values are still far from the costs associated to other physical-chemical technologies such as water scrubbing, where operating pressures of 6 − 10 bar are commonly used (Muñoz et al., 2015).

In the particular case of membrane separation technologies for biogas upgrading, the main operating costs are determined by membrane replacement (every 5–10 years) and biogas compression, since this technology operates at high pressures of up to 40 bars in gas-gas systems (Muñoz et al., 2015). However, both the vacuum pressure and the volumetric flowrate of the vacuum pump here applied (150 mbar and 1.8 Nm^3 h^{-1}, respectively) are considerably lower than those of membrane separation technologies, and therefore the increase in the operating costs due to power consumption is expected to be marginal compared to the biogas compression costs through the SC. On the other hand, a long-term operation of the degassing membrane unit coupled to the HRAP-SC system is necessary to determine the cleaning requirements and ultimately estimate membrane lifetime.

3.4 Conclusions

Novel operational strategies to reduce the N$_2$ and O$_2$ concentrations in the biomethane obtained via photosynthetic biogas upgrading were evaluated. The installation of a hollow fiber PDMS to efficiently remove DO from the cultivation broth supported DO-REs ≥ 60 % at 100 − 300 mbar of vacuum pressure, confirming the potential of membrane technology to reduce both O$_2$ and N$_2$ content in the upgraded biogas (~0.8 and ~0.1 %, respectively). The second operational strategy revealed an enhanced removal of CO$_2$ and H$_2$S when increasing the operating pressure in the SC. Unfortunately, minimum overpressures of 2 atm were necessary to limit N$_2$ and O$_2$ desorption. Under this operating condition, N$_2$ and O$_2$ concentrations of ~1.7 and ~0.1 %, respectively, were recorded in the upgraded biomethane.

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Appendix A. Supplementary data

Strategies for N₂ and O₂ removal during biogas upgrading in a pilot algal-bacterial photobioreactor

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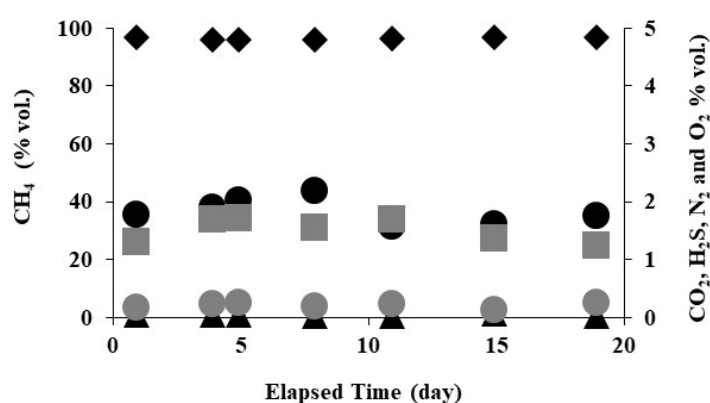
Fig. S.1. Time course of the biomethane composition prior hollow fiber PDMS membrane installation: CH₄ (dark diamonds), CO₂ (dark circle), H₂S (dark triangle), N₂ (grey square) and O₂ (grey circle).
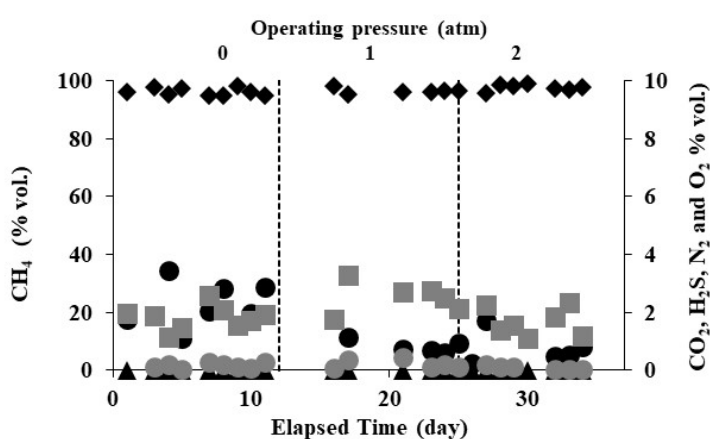
Fig. S.2. Time course of the biomethane composition during operated the pressurized scrubbing column: CH₄ (dark diamonds), CO₂ (dark circle), H₂S (dark triangle), N₂ (grey square) and O₂ (grey circle). Vertical bars represent operating at different overpressures

Optimization of photosynthetic biogas upgrading in enclosed photobioreactors combined with algal biomass production

Optimization of photosynthetic biogas upgrading in enclosed photobioreactors combined with algal biomass production

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Highlights

- Biogas upgrading and biomass production were validated in a tubular photobioreactor.

- CO_2 removals ~96 % and a complete H_2S abatement were achieved at ~1700 mg L^{-1}.

- The enclosed photobioreactor ensured a biomethane quality complying with EU standards.

- Cyclic N-deprivation increased intracellular carbohydrates storage by 2 times.

Graphical abstract
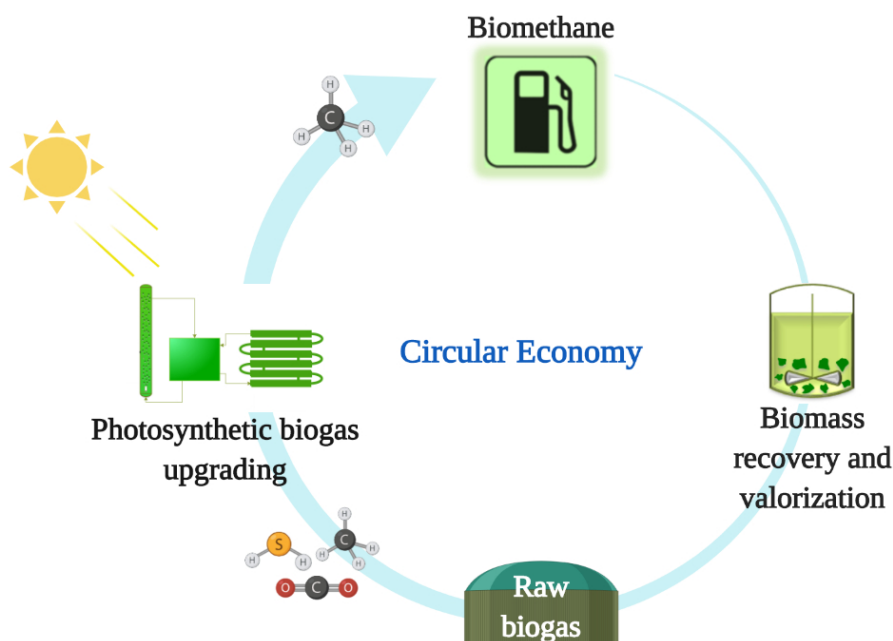
Abstract

Photosynthetic biogas upgrading in microalgae photobioreactors constitutes a sustainable and environmentally friendly technology for the simultaneous removal of CO_2 and H_2S from raw biogas. The potential of algal-bacterial symbiosis for biogas purification has been already studied and demonstrated in open photobioreactors. However, few studies have focused on the implementation of this process in enclosed photobioreactors, which offer higher photosynthetic efficiencies, enhanced biomass productivities, a limited N_2 contamination and improved CO_2 mass transfer. In this study, the influence of alkalinity (inorganic carbon concentrations from ~200 to ~1700 mg L^{-1}), and nitrogen (N) deprivation strategies (24 or 48 h limitation) on both biomethane quality and biomass composition has been evaluated and optimized in an enclosed tubular photobioreactor (Tubular-PBR) interconnected to a mixing chamber and a biogas scrubbing column (total working volume of 132 L). The increase in the alkalinity of the cultivation broth mediated an increase in the upgrading performance of the Tubular-PBR, reaching CO_2 removals of 96.3 ± 0.8 %, and a complete H_2S abatement, resulting in an enhanced biomethane composition (CH_4 = 97.2 ± 0.9 %). Moreover, process operation under N deprivation did not affect the quality of biomethane, which complied with standards required for injection into natural gas grids or use as vehicle fuel. N-deprivation promoted the continuous storage of intracellular carbohydrates (29.0 ± 4.4 % compared with 14.3 ± 2.1 % obtained under N excess). A maximum biomass productivity obtained was of 15.8 ± 4.3 g m^{-2} d^{-1} during N excess vs. 11.2 ± 3.3 g m^{-2} d^{-1} during N-deprivation at 26.8 d of hydraulic retention time. During the N feast-famine cycles, the cyanobacterial species *Pseudanabaena* sp. was dominant over green algae.

Keywords: *Alkalinity, Biogas upgrading, Biomethane, Carbohydrate production, Enclosed photobioreactor*

4.1 Introduction

Biogas is obtained from the anaerobic digestion of organic feedstock and can be used as an energy vector to produce electricity and heat in industrial and domestic applications, thus contributing to the reduction of the world dependence on fossil fuels (Sahota et al., 2018). In this context, the global installed biogas capacity reached 17.7 GW in 2018, of which 12.3 GW corresponded to Europe (IRENA, 2019). Nevertheless, a preliminary purification stage is strongly recommended prior energy use of this renewable energy source, since raw biogas contains not only methane (CH_4, 40 – 75 %), but also other gas pollutants such as carbon dioxide (CO_2, 15 – 60 %), hydrogen sulphide (H_2S, 0.005 – 3 %), nitrogen (N_2, 0 – 2 %), oxygen (O_2, 0-1 %), siloxanes (0 – 0.02 %), volatile organic compounds (VOC, < 0.6 %), ammonia (NH_3, < 1 %) or carbon monoxide (CO, < 0.6 %) (Ryckebosch et al., 2011). In this sense, the removal of CO_2 and H_2S is typically performed in order to increase the calorific value of biogas and to reduce transportation costs, besides minimizing toxicity and preventing corrosion in piping or compressors (Lebrero et al., 2016). Moreover, if the upgraded biogas is intended to be injected into the natural gas grid or to be used as a vehicle fuel, a more stricter purification is required in order to fulfill with international biomethane standards, typically $CH_4 \geq 90 – 95$ %, $CO_2 \leq 2 – 4$ %, $O_2 \leq 1$ %, and negligible amounts of H_2S

(Muñoz et al., 2015; Ryckebosch et al., 2011). In this context, physical-chemical biogas upgrading technologies have been developed and commercialized at industrial scale, water scrubbing and membrane separation being nowadays the market-leading technologies worldwide despite their high energy consumption, operating costs and environmental impacts (Awe et al., 2017; EBA, 2018).

Photosynthetic biogas upgrading in algal-bacterial photobioreactors has been consistently proven as a sustainable and environmentally friendly alternative for the simultaneous removal of CO_2 and H_2S from raw biogas in a single-stage process. This technology is based on the fixation of CO_2 by microalgae via photosynthesis and the concomitant release of oxygen to the cultivation broth, which is subsequently used by sulphur-oxidizing bacteria for the oxidation of H_2S to SO_4^{2-}. Both gases are transferred from the raw biogas to the cultivation broth in a biogas scrubbing column (SC) interconnected to the photobioreactor (Bahr et al., 2014; Muñoz et al., 2015). Several authors have evidenced the efficiency of this technology indoors and outdoors at both laboratory and pilot-scale, and the most relevant operating parameters of this technology have been optimized in order to maximize the quality of the final biomethane (Posadas et al., 2017b; Rodero et al., 2019; Serejo et al., 2015). For instance, Rodero et al., (2018b) demonstrated the key role of the alkalinity

of the cultivation broth to ensure a satisfactory biomethane quality (> 98 % of CH_4 at inorganic carbon concentrations IC > 1500 mg L^{-1}). Similarly, the influence of gas-liquid flow ratio (L/G) and configuration in the SC has been thoroughly assessed, with optimum values of ~0.5 under co-current operation (Toledo-Cervantes et al., 2017b).

Nevertheless, the economic viability of the energy valorization of biogas is often limited by the high costs of combined heat and power production gas engines and the current reductions of fiscal incentives (Wellinger et al., 2013). Similarly, the costly purification required for injection of the upgraded biomethane into natural gas grids or use as vehicle fuel renders it uncompetitive compared with natural gas. In this context, photosynthetic biogas upgrading offers not only a low-cost alternative for biogas upgrading, but also the possibility to further valorize the algal biomass generated when implemented in enclosed tubular photobioreactors (Tubular-PBR), thus increasing the economic sustainability of the technology. Under nutrient deprivation conditions, microalgae and cyanobacteria can accumulate intracellular energy reserves such as biopolymers, carbohydrates (starch in microalgae and glycogen in cyanobacteria), lipids, proteins or pigments, among others (Ángeles et al., 2019; Martín-Juárez et al., 2017; Singh and Mallick, 2017). Unfortunately, the potential of biogas upgrading has been mainly investigated and demonstrated in open photobioreactors such as high rate algal ponds (HRAPs) due to their simple design, construction, operation, easy scale-up and lower investment cost (Alcántara et al., 2015). In contrast, this configuration offers a lower biomass productivity as a result of the reduced light photosynthetic efficiency compared to enclosed photobioreactors (Park et al., 2011; Posten, 2009).

Therefore, photosynthetic biogas upgrading combined with algal biomass valorization in enclosed photobioreactors constitutes a promising cost-efficient and sustainable alternative that integrates a reduction in the costs of biogas upgrading within a model of circular economy. However, there is a lack of studies devoted to assess and optimize the long-term performance of this technology. In this study, the biogas upgrading performance of a Tubular-PBR interconnected to an external SC was evaluated for 395 days, systematically assessing the influence of the alkalinity of the cultivation broth on the biomethane quality. Moreover, the effect of nitrogen (N) deprivation cycles on biogas upgrading and on the chemical composition of the algal biomass were determined in order to validate biomass valorization.

4.2   Materials and methods

4.2.1 Inoculum and culture conditions

The Tubular-PBR was inoculated with a microalgae-bacteria consortium from an outdoors HRAP treating domestic wastewater (Chiclana de la Frontera Wastewater Treatment Plant, Spain). A modified Brunner medium was employed during the operation of the Tubular-PBR. This medium was composed of (g L^{-1}): Na_2HPO_4, 2.44; KH_2PO_4, 1.52; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.20; $CaCl_2 \cdot 2H_2O$, 0.05, and 5 mL of SL-4 stock solution. The SL-4 stock solution contained (g L^{-1}) EDTA, 0.5; $FeSO_4 \cdot 7H_2O$, 0.2, and 100 mL of SL-6 stock solution with a composition of (g L^{-1}): $ZnSO_4 \cdot 7H_2O$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.03; H_3BO_3, 0.30; $Co(NO_3)_2 \cdot 6H_2O$, 0.20; $Na_2MoO_4 \cdot 2H_2O$, 0.03; $CuCl_2 \cdot 2H_2O$, 0.01; $NiCl_2 \cdot 6 \ H_2O$, 0.02. All chemicals were purchased from Panreac® (Barcelona, Spain) with a purity of at least 99 %. Inorganic carbon was added as a mixture of $NaHCO_3$ and Na_2CO_3 (purchased from Cofarcas S.A. (Burgos, Spain)) at a concentration that varied depending on the operational stage.

4.2.2 Experimental set-up

The experimental set-up consisted of a Tubular-PBR interconnected to a mixing chamber and a SC, with a total working volume of 132 L (Fig. 4.1). The Tubular-PBR was composed of 12 tubes of 6 cm of inner diameter and 94 cm of length, with a total volume of 45.6 L, an illuminated area of 2.2 m^2 and a total footprint of 0.3 m^2. Two sets of high intensity LED PCBs were placed at both sides of the Tubular-

PBR to provide a photosynthetic active radiation (PAR) of ~883 µmol m^{-2} s^{-1} using 12:12 h light:dark periods. The mixing chamber (50 cm height, 48 cm width and 35 cm length) had a working volume of 84 L, and the SC (2 m height with an internal diameter of 4.5 cm) was operated with 1.73 m of water column, and a working volume of 2.6 L. The cultivation broth was recirculated using a centrifugal pump (DWO/E 300, EBARA, Italy) at a linear velocity of 0.5 m s^{-1} through the Tubular-PBR and the mixing chamber. Flue-gas or biogas was supplied to the SC through a stainless-steel diffuser of 2 µm pore size located at the bottom of the column, co-currently with the cultivation broth from the mixing chamber.

4.2.3 Operational procedure

4.2.3.1 Influence of IC concentration on photosynthetic biogas upgrading

The system was inoculated with the microalgae-bacteria consortium at a volatile suspended solids (VSS) concentration of 1.6 g L^{-1}, and initially operated in continuous mode for 39 days for inoculum acclimation. During this initial period, 3.1 L of the cultivation broth were daily exchanged with fresh mineral salt medium until reaching a steady biomass productivity of 13.0 ± 0.6 g m^{-2} d^{-1}, calculated according to footprint (Posten, 2009). Neither flue-gas nor biogas were supplemented to the SC during the acclimation period. Afterwards, the

system was operated for 395 days at a cultivation broth exchange rate of 5 L d^{-1} (corresponding to a hydraulic retention time (HRT) of 26.8 d).

During Stage 0, synthetic flue-gas (CO$_2$ 30 % and N$_2$ 70 % v/v, Abelló Linde, Spain) was used as carbon source for the growth of microalgae. From days 0 – 14, synthetic flue-gas was fed at 10 mL min^{-1}, subsequently increasing the flow rate to 20 mL min^{-1} for 53 days. From day 67 onwards, a synthetic biogas mixture (CH$_4$ 70 %, CO$_2$ 29.5 %, and H$_2$S 0.5 % v/v, Abelló Linde, Spain) was fed at 20 mL min^{-1}, corresponding to a L/G ratio of 0.5. In order to test the influence of cultivation broth alkalinity on biomethane quality, the IC concentration was increased from 218.8 ± 5.1 to 429.5 ± 47.6 and 1707.3 ± 52.8 mg L^{-1} by addition of NaHCO$_3$ and Na$_2$CO$_3$ in Stages I, II, and III, respectively (Table 4.1). During Stages IV to VI, the IC concentration was maintained constant at 1347.9 ± 54.4 mg L^{-1}.

4.2.3.1 Influence of the cyclic N deprivation on biomass composition

During Stages 0, I, II, and III, 529.5 mg N d^{-1} (corresponding to 1 g (NH$_4$)$_2$SO$_4$ L^{-1}) were added with the fresh mineral medium in order to maintain a total nitrogen (TN) concentration of 87.2 ± 11.9 mg L^{-1} in the cultivation broth. By day 191 (Stage IV), fresh mineral medium without N was supplemented in order to decrease the TN concentration to ~6 mg L^{-1} by day 216,

starting afterwards the N feast-famine cycles. During Stages IV and V, 24- and 48-hours feast-famine cycles were implemented, respectively, consisting of the exchange of N-supplemented medium by day 1 of the cycle, N-free medium by day 2 and no medium exchange by day 3 (in Stage V). N concentration in the N-supplemented mineral medium was adjusted to provide 324 mg N d^{-1} during the feast periods (0.6 g (NH$_4$)$_2$SO$_4$ L^{-1}) to ensure a biomass productivity of ~13 g m^{-2} d^{-1}. Finally, by day 283 (Stage VI), N supplementation was restored and 529.5 mg N d^{-1} were added with the fresh mineral medium to reach a TN concentration of 81.8 ± 4.5 mg L^{-1} in the cultivation broth (Table 4.1).

Gas samples from the inlet and outlet of the biogas SC were daily drawn to monitor the concentration of CH$_4$, CO$_2$, H$_2$S, N$_2$, and O$_2$. Liquid samples of the cultivation broth were collected twice a week from the mixing chamber in order to analyze the pH, temperature, dissolved oxygen (DO), biomass (as volatile suspended solids, VSS), TN, IC, total organic carbon (TOC), nitrite (NO$_2^-$), nitrate (NO$_3^-$), phosphate (PO$_4^{3-}$), and sulphate (SO$_4^{2-}$) concentration. An aliquot of 1.5 mL of algal-bacterial biomass was drawn in each steady-state for the determination of the microalgae population structure. Additionally, liquid samples for the determination of TN, NO$_3^-$, PO$_4^{3-}$, and SO$_4^{2-}$ concentration and biomass characterization (carbohydrates, lipids, and proteins content) were daily drawn

before mineral medium exchange during Stages IV, V, and VI. Additionally, liquid samples were collected after 1 hour following fresh mineral medium addition in order to monitor the concentration of TN and anions.
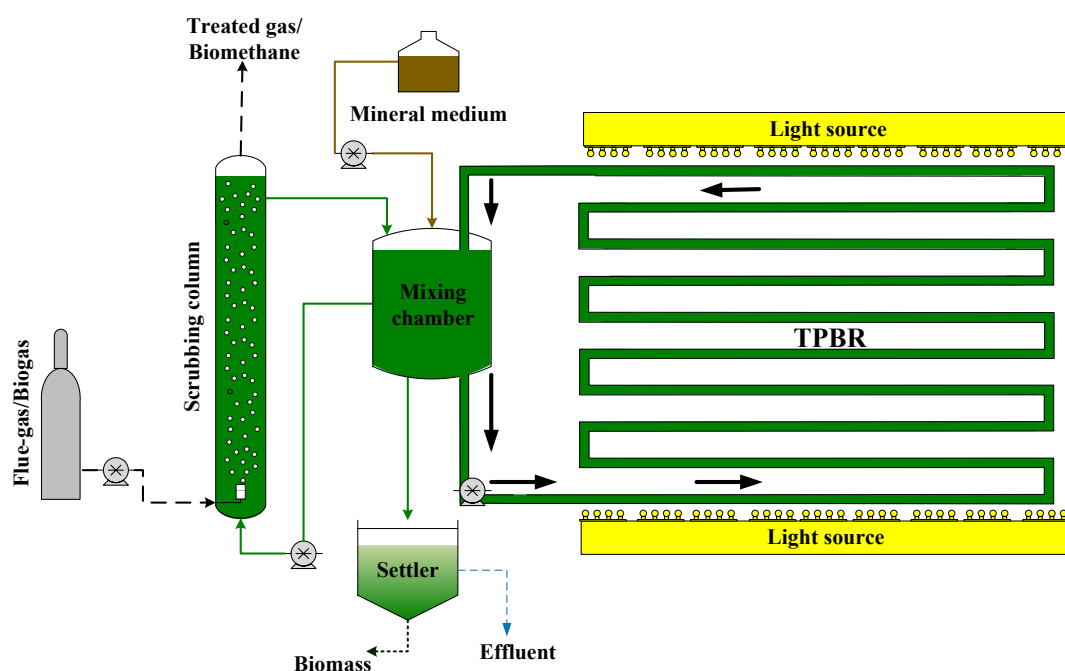

Fig. 4.1 Schematic diagram of the experimental set-up used for photosynthetic biogas upgrading.

Table 4.1 Average values of the operating parameters in the cultivation broth under steady state conditions during the different operational stages.

Stage	Days	Operational strategy	Gas phase	pH	DO (mg O_2 L^{-1})	TN (mg N L^{-1})	N-NO$_3^-$ (mg N L^{-1})	N-NO$_2^-$ (mg N L^{-1})	TOC (mg L^{-1})	IC (mg L^{-1})	$T_{Tubular-PBR}$ (°C)
0	0–66	-	Flue gas	9.2 ± 0.3	18.9 ± 2.9	76.9 ± 11.0	72.1 ± 20.1	4.1 ± 5.7	48.3 ± 26.6	217.1 ± 29.3	30.4 ± 1.1
I	67–99	Influence of IC concentration	Biogas	8.9 ± 0.2	20.4 ± 3.0	102.2 ± 2.0	112.1 ± 12.8	0.0 ± 0.0	9.6 ± 5.8	218.8 ± 5.1	26.7 ± 3.1
II	100–117	Influence of IC concentration	Biogas	9.2 ± 0.2	18.4 ± 2.7	97.1 ± 3.9	102.3 ± 20.8	0.0 ± 0.0	19.2 ± 7.9	429.5 ± 47.5	29.1 ± 1.8
III	118–190	Influence of IC concentration	Biogas	9.3 ± 0.1	17.6 ± 5.3	77.2 ± 8.5	85.6 ± 11.3	2.9 ± 3.9	49.5 ± 12.8	1707.3 ± 52.8	26.4 ± 1.3
IV	191–240	24-h feast-famine cycles	Biogas	9.8 ± 0.1	11.2 ± 3.5	4.9 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	40.3 ± 26.3	1285.1 ± 56.6	28.3 ± 1.4
V	241–282	48-h feast-famine cycles	Biogas	9.6 ± 0.1	14.0 ± 5.3	6.2 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	112.0 ± 83.2	1379.0 ± 87.9	30.9 ± 3.5
VI	283–395	N supplementation	Biogas	9.4 ± 0.1	11.3 ± 4.4	92.7 ± 6.3	81.8 ± 4.5	3.6 ± 2.0	152.2 ± 73.8	1379.5 ± 47.8	27.3 ± 1.4

DO: dissolved oxygen, TN: total nitrogen, TOC: total organic carbon, IC: inorganic carbon.

4.2.4 Analytical procedures

4.2.4.1 Biogas and biomethane composition

Biogas and biomethane composition (CH_4, CO_2, H_2S N_2, and O_2) was analyzed by gas chromatography in a Bruker 430 GC-TCD (Bruker, Palo Alto, USA) equipped with a CP-Molsieve 5A (15 m × 0.53 mm × 15 µm) and a CP-PoraBOND Q (25 m × 0.53 mm × 10 µm) columns. The oven, injector and detector temperatures were maintained at 45, 150, and 200 °C, respectively. Helium was used as the carrier gas at 13.7 mL min^{-1}

4.2.4.2 Chemical biomass composition characterization

Quantitative determination of the carbohydrates was carried out by adapting the phenol-sulfuric acid method (Dubois et al., 1956). Aliquots of 1.5 mL of biomass were harvested from the cultivation broth by centrifugation (10000 rpm, 10 min). The supernatant was discarded and 4 mL of H_2SO_4 (1 M) were added to the pellet prior incubation for 20 min at 100 °C in a thermoreactor TR 300 (Merck KGaA, Darmstadt, Germany). After digestion, the solution was centrifuged for 5 min at 10000 rpm, 0.5 mL of the supernatant were mixed with 0.5 mL of 5 %$_{w/v}$ phenol solution and the mixture incubated for 40 min. Finally, 2.5 mL of H_2SO_4 (97 %) were added to the mixture and the absorbance was measured at 485 nm in a spectrophotometer (Shimadzu, UV-2550, Japan). D-glucose was used as standard.

The protein and lipid content were determined using lyophilized biomass at -80 °C (LyoQuest 55 Plus, Telstar®, Spain). The protein content was measured by Kjeldahl Total Nitrogen (KTN), multiplying the result by a factor 5.95. Glass tubes containing 100 mg samples of dry biomass, 25 mL of distilled water, Kjeldahl catalyst (Cu) (0.3 % in $CuSO_4 \cdot 5H_2O$), and 6 mL of H_2SO_4 (97 % purity) were placed in a KTN digestion block (Bloc digest 12, JP Selecta SA, Spain). The temperature was initially set at 150 °C for 20 minutes, increased then to 380 °C and maintained for 1 hour. After acid digestion, the samples were distilled in a KjelFelex K-360 (Buchi, Switzerland) using a boric acid solution and NaOH 6 N. Finally, the borate solution was titrated with a standard acid solution (H_2SO_4 0.1 N). The nitrogen content was calculated from the relation of the volume of H_2SO_4 required to neutralize, and the dry weight of algal biomass (Owusu-apenten, 2002).

Aliquots of 100 mg of biomass, 100 mg Al_2O_3 and 2 mL of a solution of chloroform in methanol (2:1 v/v) were used for lipid extraction. The samples were centrifuged at 3600 rpm (SIGMA 2-16P, SIGMA® Laborzentrifugen GmbH, Germany) for 3 minutes. The supernatant was carefully withdrawn, and this procedure was repeated until a light-colored organic phase was obtained. Then, 3 mL of HCl 0.1 N and 0.3 mL of a solution of 0.5 % $MgCl_2$ were added to the organic phase and

centrifuged. The organic phase was then separated and lipid content was quantified gravimetrically (Gary Kochert, 1978).

Finally, the moisture and ash content were estimated from a sample of 100 mg of freeze-dried algal biomass. A melting crucible was preconditioned at 550 °C overnight (CARBOLITE AAF 1100, CARBOLITE® GERO, Germany), left to cool to room temperature in a desiccator, and weighted. The sample was then dried at 105 °C for 24 hours, allowed to cool to room temperature in a desiccator, and weighted. Next, the dried samples were dry ashed at 550 °C in the muffle for 24 hours and cooled to room temperature in a desiccator. The constant weight of the dry ash samples was recorded. The moisture and ash percentages were calculated according to equations 4.1 and 4.2, respectively (Van Wychen and Laurens, 2016).

$$\% \, moisture = 100 - \left[\frac{(W_{dried\,biomass} - W_{crucible})}{W_{freeze-dried\,biomass}} \times 100 \right] \quad \text{(Eq. 4.1)}$$

$$\% \, ash = 100 - \left[\frac{(W_{dried\,biomass} - W_{ash\,biomass})}{W_{freeze-dried\,biomass}} \times 100 \right] \text{(Eq. 4.2)}$$

Chemical biomass composition was expressed as percentage dry cell weight (% dcw).

4.2.4.3 Cultivation broth characterization

Dissolved IC, TOC and TN concentrations were determined in the cultivation broth using 30 mL of filtered sample (0.45 µm) via a Shimadzu TOC-VCSH analyzer (Japan)

equipped with a TNM-1 chemiluminescence module. NO_2^-, NO_3^-, PO_4^{3-} and SO_4^{2-} concentrations were measured by HPLC-IC in 1.5 mL of sample was filtered (0.22 µm) using a Waters 515 HPLC pump coupled with an ion conductivity detector (Waters 432) and equipped with an IC-PAK Anion HC column (4.6 mm × 150 mm) and an IC-Pak Anion Guard-Pak (Waters). TSS and VSS concentration was determined from the cultivation broth using 20 mL filtered samples (0.45 µm) according to Standard Methods (APHA, 2005). The DO concentration was monitored by an Oxi 3310 connected to a sensor CellOx 325 (WTW, Germany), while the pH was analyzed with a Eutech Cyberscan 510 (Eutech Instruments, The Netherlands). The PAR at the Tubular-PBR surface was recorded with a LI-250A light meter (LI-COR Biosciences, Germany). Microalgae identification was carried out after fixation with 5 % of lugol and 10 % formaldehyde, using the standard technique described by Utermöhl, (1958). In this sense, 50 mL cylindrical tubes connected to a glass sedimentation chamber were used for cell counting. Each sample was previously homogenized both manually and using a glass homogenizer. The chamber was left on a flat surface to ensure that the sedimentation of the sample was completed. Afterward, a glass coverslip was positioned at the sedimentation chamber and cells were counted and identified under a Nikon IK70 inverted photonic microscope according to Sournia, (1978). Taxonomic identification

reached the genus or species level, according to literature (Susswasserflora von Mitteleuropa). Specific abundances were calculated according to equation 4.3.

$$\frac{Cells}{mm^2} = \frac{C \times A_{tf} \times D_f \times V_t}{FA \times N_{fs} \times V_{sc} \times A_{ts}}$$ (Eq. 4.3)

Where C = Total cells counted; A_{tf} = Area of the total fields on microscope; D_f = Dilution factor of the sample; V_t = Volume of the total sample; FA = Microscopic field area; N_{fs} = Number of microscopic random fields scanned; V_{sc} = Volume of subsample used for counting; A_{ts} = Initial volume.

4.3 Results and discussion

4.3.1 Influence of IC concentration on system performance

4.3.1.1 *Biogas upgrading performance (Stages I-III)*

By day 34 after system inoculation, CO_2 removal efficiencies (REs) of 74.0 ± 4.9 % were recorded during flue gas feeding (Stage 0) at an inorganic carbon concentration in the cultivation broth of 217.1 ± 29.3 mg L^{-1}. When biogas was supplemented to the SC, average CO_2-REs increased from 77.7 ± 3.9 to 85.4 ± 3.3, and 96.3 ± 0.8 % at IC concentrations of ~200, 400, and 1700 mg L^{-1} in Stages I, II, and III, respectively (Fig. 4.2a). During these stages, almost a complete H_2S removal (H_2S-REs > 99 %, Fig. 4.2a) was achieved due to the higher aqueous

solubility of H_2S compared to that of CO_2, according to Henry's Law constants at 20 °C (H_{H2S} ≈ 2.13 and H_{CO2} ≈ 0.71, dimensionless) (Sander, 2015). This removal was attributed to the activity of sulphiide oxidizing bacteria using the oxygen from the photosynthetic activity, as supported by the values of sulfate concentration recorded throughout the experimental period (890.0 ± 5.7 mg SO_4^{2-} L^{-1}).

The pH of the cultivation broth remained at 8.9 ± 0.2, 9.2 ± 0.2, and 9.3 ± 0.1, during Stages I, II, and III, respectively. The positive effect of alkalinity on the removal of both acidic gases has been widely demonstrated in HRAPs both at laboratory and pilot scales. However, a higher pH (> 9.4) is commonly required to achieve CO_2-REs as high as those observed in this study, while process operation at a pH ~9 typically results in a lower abatement performance (REs < 90 %) (Bose et al., 2019). Similarly, Rodero et al., (2018b) observed CO_2-REs of 50.6 ± 3.0 % when working at an IC concentration of ~500 mg L^{-1} in the cultivation broth in an open photobioreactor, considerably below the abatement performance of the enclosed system here studied. Moreover, H_2S-REs from 96 to 100 % have been reported in open systems at ~1500 mg IC L^{-1}, however, lower IC concentrations (< 500 mg IC L^{-1}) only supported H_2S-REs of 66 – 94 % (Franco-Morgado et al., 2017; Posadas et al., 2017b; Rodero et al., 2019, 2018b). The high alkalinity of the cultivation broth promotes the mass transfer of the acidic

gases CO_2 and H_2S from the biogas to the liquid phase in the SC, since the presence of carbonate/bicarbonate increases the pH of the cultivation broth.

The average CO_2 content in the upgraded biogas was 6.2 ± 1.8, 4.6 ± 1.1,
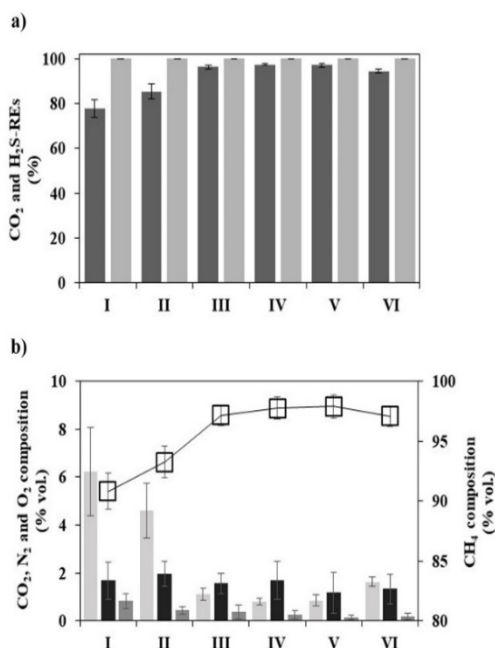

Fig. 4.2. a) Average CO_2 (dark grey bars) and H_2S (light grey bars) removal efficiencies (REs) during the different operational stages. b) Upgraded biogas composition obtained in the different operating stages: CO_2, (light grey bars), N_2, (black bars), O_2, (dark grey bars) and CH_4 (squares). Vertical bars represent data variability under steady state calculated as standard deviation (± SD, n = 6 – 22).

Previous research on photosynthetic biogas upgrading in open photobioreactors has found N_2 and O_2 concentrations in the upgraded biogas ranging from 1 to 13 %, and from 0.7 to 7 %, respectively (Meier et al., 2018; Posadas et al., 2015a, 2017b). Thus, in open

and 1.1 ± 0.2 % during Stages I, II, and III, respectively, while no H_2S was detected in the biomethane during these operating stages (Fig. 4.2b). Consequently, an average CH_4 concentration of 90.8 ± 1.5, 93.3 ± 1.3, and 97.2 ± 0.9 % (Stages I, II, and III, respectively) was achieved. On the other hand, the average N_2 concentration in the biomethane was 1.7 ± 0.8, 2.0 ± 0.5, and 1.6 ± 0.4 %, with an O_2 content of 0.8 ± 0.3, 0.5 ± 0.2, and 0.4 ± 0.3 %, during Stages I, II, and III, respectively. Hence, the optimum biomethane composition was recorded during Stage III working at ~1700 mg IC L^{-1}, which indeed complied with most international regulations for biogas injection into natural gas grids or use as vehicle fuel (CH_4 ≥ 95 %, CO_2 ≤ 2.5 – 4 %, H_2S < 5 mg Nm^{-3}, and O_2 < 1 %) (Muñoz et al., 2015).

systems, the high photosynthetic activity results in high DO concentrations and a subsequent increase in the O_2 content in the biomethane (Meier et al., 2017). In contrast, in our Tubular-PBR, the limited stripping from the cultivation broth in the SC allowed achieving satisfactory O_2 contents in the upgraded biogas in spite of the high photosynthetic activity and the concomitant high DO levels (Stages I to III, DO > 17 mg L^{-1}).

4.3.1.2 Biomass production (Stages I-III)

A high photosynthetic activity was recorded regardless of the operating stage, with DO concentrations in the

cultivation broth ranging from 17 to 20 mg O_2 L^{-1}. It is important to stress that no inhibition of the microalgae growth associated to this elevated oxygen concentration was observed, which has been reported as one of the possible drawbacks of enclosed photobioreactors (DO > 30 mg O_2 L^{-1}) (Grima et al., 1999; Kazbar et al., 2019). The low dilution rate set during Stages I-III resulted in a low biomass productivity and VSS concentration in the Tubular-PBR in Stage I (10.8 ± 2.0 g m^{-2} d^{-1} and 569.0 ± 106.3 mg L^{-1}, respectively) (Fig. 4.3), which slightly increased to 13.4 ± 1.8 and 12.1 ± 2.7 g m^{-2} d^{-1} during Stages II and III, respectively (VSS concentrations of 711.3 ± 95.6 and 697.5 ± 60.8 mg L^{-1}). Similar biomass concentrations have been recorded in open systems devoted to biogas upgrading (Bose et al., 2019). For instance, Rodero et al. (Rodero et al., 2018b) reported biomass concentrations of ~200 – 500 mg TSS L^{-1}, depending on the IC concentration in the cultivation broth of a HRAP (ranging from 100 to 1500 mg IC L^{-1}). On the contrary, biomass productivities from 5 up to 16.9 g m^{-2} d^{-1} have been obtained in HRAPs working at lower HRTs compared to that of the present study (HRT = 4 – 7.4 vs. 26.8 days) (Posadas et al., 2017b, 2015b; Rodero et al., 2018b). In this regard, it is important to highlight that volumetric (g L^{-1} d^{-1}) instead of areal productivities (g m^{-2} d^{-1}) should be reported for enclosed photobioreactors to allow for a fair comparison between both configurations (Chini Zittelli et al., 2006; Posten, 2009). Thus, volumetric productivities of 0.08 – 0.07 g L^{-1} d^{-1} were obtained in Stages II and III, at the high end of those commonly achieved in open systems ranging from 0.015 to 0.1 g L^{-1} d^{-1} (Toledo-Cervantes et al., 2018).

Finally, $N-NH_4^+$ was completely oxidized into nitrate by nitrifying bacteria in Stages I and II (Table 4.1). Indeed, $N-NO_3^-$ concentrations of 112.1 ± 12.8, 102.3 ± 20.8, and 85.6 ± 11.3 mg L^{-1} were recorded in Stages I, II, and III, respectively. Negligible $N-NO_2^-$ concentration of 2.9 ± 3.9 mg $N-NO_2^-$ L^{-1} was detected in the cultivation broth of the Tubular-PBR during Stage III.
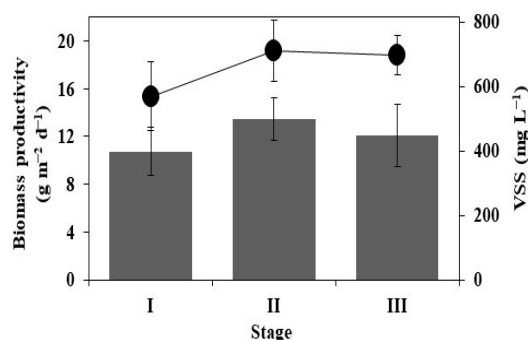

Fig. 4.3. Influence of the inorganic carbon (IC) concentration on biomass productivity (g m^{-2} d^{-1}) (grey bars) and volatile suspended solids (VSS) concentration (dark circles). Vertical bars represent data variability under steady state calculated as standard deviation (± SD, n = 8 – 15).

4.3.2 Effect of cyclic N-deprivation on the system performance (Stages IV to VI)

4.3.2.1 Biogas upgrading performance

During Stages IV to VI, the IC concentration in the cultivation broth remained constant at ~1300 mg L^{-1}, while a slightly higher pH was recorded compared to that of N supplementation stages, with average values of 9.8 ± 0.1, 9.6 ± 0.1, and 9.4 ± 0.1 in Stages IV, V, and VI, respectively. This higher pH concomitant with the lower DO concentrations presents in the Tubular-PBR (11.2 ± 3.5, 14.0 ± 5.3, and 11.3 ± 4.4 mg L^{-1} in Stages IV, V, and VI, respectively) was likely due to the absence of a significant nitrification during Stages IV and V. Indeed, NH_4^+ nitrification is a biological process that decreases the pH of the cultivation broth as a result of proton release and IC consumption.

Under these operational conditions, no H_2S was detected in the biomethane and CO_2-REs of 97.3 ± 0.5, 97.0 ± 0.8, and 94.5 ± 0.8 % were recorded in Stages IV, V, and VI, respectively. The superior CO_2 removals observed during N-deprivation were in agreement with the more alkaline pH required for an effective mass transfer of this contaminant from biogas into the cultivation broth. In this context, an enhanced upgraded biogas composition was obtained: CH_4 = 97.7 ± 1.0 %, CO_2 = 1.0 ± 0.4 %, H_2S = 0.0 ± 0.0 %, O_2 = 0.2 ± 0.1 %, and N_2 = 1.3 ± 0.8 % (Fig. 4.2b).

4.3.2.2 Biomass production

During the operation of the Tubular-PBR under N-deprivation conditions, a biomass productivity of 12.4 ± 1.7, and 11.2 ± 3.3 g m^{-2} d^{-1} was recorded in Stages IV and V, respectively, corresponding to a VSS concentration of 654.2 ± 90.2, and 590.4 ± 176.8 mg VSS L^{-1} (Table 4.2). This decrease in biomass concentration was in accordance with the reduction in the N load to the photobioreactor. On the other hand, a recovery in the biomass productivity and VSS concentration up to values of 15.8 ± 4.3 g m^{-2} d^{-1} and 779.3 ± 307.3 mg L^{-1} was observed when the nitrogen feed was restored (Stage VI, 529.5 mg N d^{-1}). Nevertheless, the photosynthetic performance of microalgae was not negatively affected as shown by the DO concentration of the cultivation broth, which was maintained within the optimal growth range (> 10 mg L^{-1}).

The ability of microalgae to accumulate energy storage compounds under different nutrient availability conditions can contribute to the economic viability of the photosynthetic upgrading process. In this context, a highly variable composition of proteins (40 – 60 %), lipids (5 – 20 %), carbohydrates (20 – 30 %), and ashes (5 – 15 %) can be obtained depending on the microalgae species (Ángeles et al., 2019). As expected, the microalgae consortium of the Tubular-PBR exhibited a higher protein content under N excess compared to that observed under N limitation: ~33 – 37 % dcw in

Stages IV and V *vs.* ~48.5 % dcw in Stage VI (Table 4.2). Besides, previous studies have demonstrated an enhanced nitrogen uptake associated with an increased protein production when increasing the inorganic carbon concentration available for microalgae growth (White et al., 2013).

Regarding lipids accumulation, values of 10.4 ± 1.5 and 9.7 ± 3.1 % dcw were obtained during N-deprivation in Stages IV and V, respectively, unexpectedly increasing to 17.0 ± 1.2 % dcw in the last operating stage when N was supplemented in excess. The lipid yield here obtained was significantly low compared to the typical ranges of lipid content commonly found in microalgae (from 20 up to 70 % dcw), and therefore not suitable for further valorization (Ángeles et al., 2019; Koutra et al., 2018). Similarly, Posadas et al., (2016) obtained a lipid content ranging from 2.9 to 11.2 % dcw in microalgae grown in a HRAP devoted to biogas upgrading. In this context, it is important to highlight that the IC contained in the cultivation broth is not only an essential parameter regulating photosynthesis and microalgae growth, but it might also influence the biochemical composition of the biomass. In this sense, some authors have reported an increased carbohydrates or lipids accumulation depending on the microalgae strain when N-deprivation occurs under excess of carbon source (Kassim and Meng, 2017; Rueda et al.,

2020; White et al., 2013). In our particular study, N deprivation mediated an increase in the accumulation of carbohydrates in the microalgae biomass, achieving values up to 2.0 times higher (29.0 ± 4.4 % dcw in Stage V) than those obtained under regular nutritional conditions. This might support alternative chemical or biological valorization strategies for this biomass. Therefore, the synthesis of lipids typically reported under N limitation was here diverted to carbohydrates synthesis during CO_2 fixation in the Calvin-Bassham-Benson cycle (CBB). The glyceraldehyde–3–phosphate (G3P) produced through the CBB cycle can either be used for the synthesis of pyruvate via the glycolytic pathway, which is the precursor for the synthesis of amino acids, lipids, pigments, and proteins; or for the synthesis of pigments or carbohydrates (Fig. 4.4) (Liang et al., 2019; Perez-Garcia et al., 2011).

In brief, the results here obtained demonstrated the possibility of tailoring the biochemical composition of microalgae by implementing operational strategies based on sequential N limitations. Nevertheless, the adaptation of the microalgae population to the environmental conditions, as discussed in the next section, plays a key role and hinders a proper control of the productivity of the target bioproduct.

Table 4.2. Effect of N feast-famine cycles on the areal biomass productivity and chemical composition: carbohydrates, lipids, and proteins. Values are presented as the steady state average with the corresponding standard deviation (± SD, n = 3 − 27).

Stage	IV (24 h N-famine cycles)	V (48 h N-famine cycles)	VI (regular nutrition)
Areal biomass productivity ($g\ m^{-2}\ d^{-1}$)	12.4 ± 1.7	11.2 ± 3.3	15.8 ± 4.3
Chemical composition (% dcw)			
Carbohydrates	24.7 ± 5.9	29.0 ± 4.4	14.3 ± 2.1
Lipids	10.4 ± 1.5	9.7 ± 3.1	17.0 ± 1.2
Proteins	33.2 ± 2.5	37.4 ± 1.2	48.7 ± 1.7
Ashes	20.6 ± 2.7	18.1 ± 1.0	16.6 ± 0.4
Moisture	8.9 ± 2.7	3.4 ± 0.9	2.5 ± 0.4
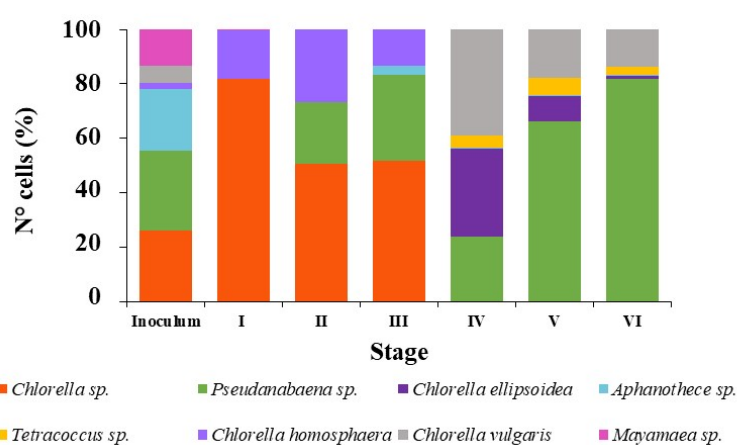
Fig. 4.5. Microalgae population structure in the Tubular-PBR during the different operational stages.

4.3.3 Microalgae population structure

The Tubular-PBR was inoculated with a cyanobacteria/microalgae consortium composed of *Aphanothece* sp., *Chlorella* sp., *Chlorella vulgaris*, *Mayamaea* sp., *Chlorella homosphaera*, and *Pseudanabaena* sp. (with a share of 21, 24, 6, 12, 2, and 27 % Nº cells, respectively) (Fig. 4.5). Interestingly, biogas upgrading at the lowest inorganic carbon concentration supported the dominance of *Chlorella* species during Stage I.

Nevertheless, the stepwise increase in IC during Stages II and III mediated a shift in microalgae structure, decreasing the abundance of the green algae *Chlorella* sp. and *Chlorella homosphaera* from 79 to 52 %, and from 17 to 13 % Nº cells, respectively, concomitant with an increase in the abundance of the cyanobacterium *Pseudanabaena* sp. of 22, and 32 % Nº cells in Stages II and III, respectively. The contribution of this cyanobacterium increased in subsequent operational stages, being the dominant

species in Stages V (64 % N° cells), and VI (79 % N° cells). *Pseudanabaena* sp. is commonly found during wastewater treatment at low N concentrations competing with species from the genus *Chlorella* (Arias et al., 2020). Interestingly, *Tetracoccus* sp., a sulphur-oxidizing phototrophic bacteria typically found in wastewater treatment processes, was retrieved in Stages IV – VI with an abundance of up to 6 % N° cells (Yamaguchi et al., 2006). Overall, the low microalgae diversity found in this study was attributed to the high alkalinity and the N deprivation conditions.
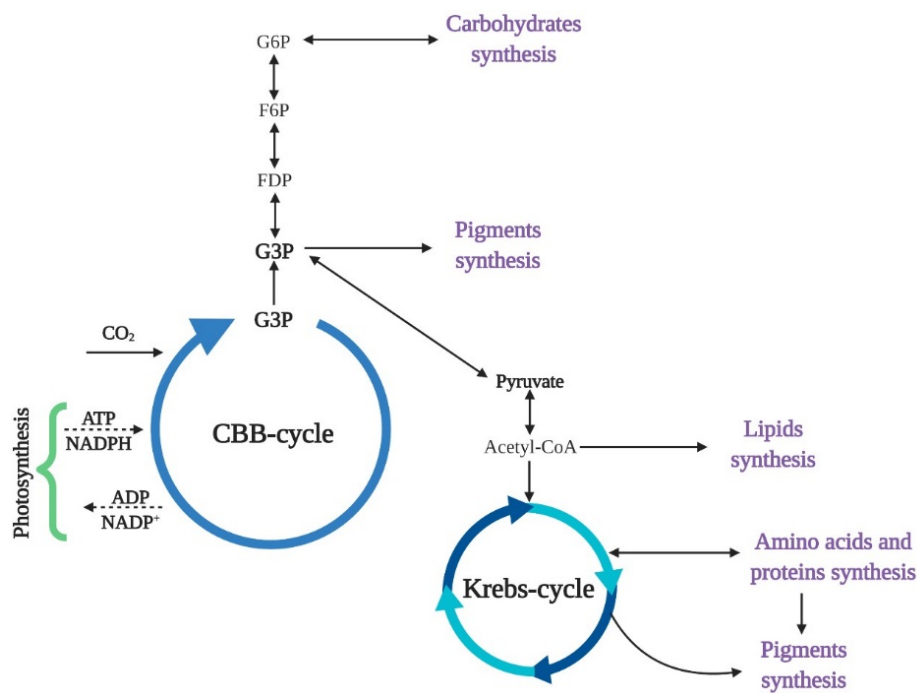
Fig. 4.4 Metabolic pathways for carbon assimilation and production of bioproducts by microalgae. ADP, adenosine-diphosphate; ATP, adenosine-triphosphate; F6P, fructose-6 phosphate; FDP, fructose 1,6-biphosphate; G6P, glucose-6-phosphate; NADP+, nicotinamide adenine dinucleotide phosphate (oxidised); NADPH, nicotinamide adenine dinucleotide phosphate (reduced); G3P, glyceraldehyde–3–phosphate. (Adapted from Perez-Garcia et al., (2011). Figure created with BioRender.com).

4.3.4 Perspectives and practical applications

As demonstrated in this study, the biomethane obtained from photosynthetic biogas upgrading complies with most international regulations for injection into the natural grid or use as vehicle fuel. Nevertheless, the determination of the cost-benefit ratio and the techno-economic feasibility is necessary prior full-scale implementation of this technology. In this context, biogas upgrading in an enclosed algal-bacterial

photobioreactor coupled with an external SC offers a low energy consumption of ~0.08 kW-h Nm^{-3} of treated raw biogas, in contrast with the energy consumption of conventional physical-chemical technologies which ranges from 0.2 up to 1 kW-h Nm^{-3} of treated raw biogas (Toledo-Cervantes et al., 2017a). Moreover, this process allows for the simultaneous biogas upgrading (CO_2 and H_2S removal in one stage), algal biomass valorization and wastewater treatment, which could play a crucial role in a circular economy model. On the contrary, the investment costs of photosynthetic biogas upgrading are ~2 times higher than those of the market-leading technologies for biogas upgrading (Angelidaki et al., 2019; Toledo-Cervantes et al., 2017a). Currently, European Commission projects such as INCOVER (https://incover-project.eu/) or URBIOFIN (https://www.urbiofin.eu/) are validating the potential of this biogas upgrading technology at semi-industrial scale outdoors, integrating the process in an wastewater treatment plant and an urban solid waste treatment facility, respectively (Angelidaki et al., 2019).

On the other hand, the feasibility of this biological technology could be boosted by increasing the algal biomass economic value through the accumulation of high-added value bioproducts (Meier et al., 2019). In this sense, the algal biomass with an enhanced content of carbohydrates obtained in this research during the N feast-famine cycles could be used as a

feedstock to produce bioethanol. However, the biomass recovery step, which depends on factors such as cell type, density and size, alongside with the downstream processing requirements and the final value of the product, must be carefully assessed in order to ensure the viability of the process (Zabed et al., 2020). For example, the extraction of carbohydrates, lipids, and proteins was studied in a pilot-scale membrane filtration system at Swansea University, resulting in an energy consumption of 0.90 kW-h m^{-3} per 0.05 € kg $_{microalgae}^{-1}$ (Gerardo et al., 2015). Similarly, a research group from Plymouth University implemented a process consisting of auto-flocculation coupled with passive capillary dewatering for the extraction of bioactive compounds from cyanobacteria, reaching a lower energy consumption (0.01 kW-h m^{-3} per 0.16 € kg $_{microalgae}^{-1}$) (Gerardo et al., 2015). In brief, the selection of the optimal harvesting technology must be performed on a case by case basis, taking into account microalgae species, high end added value products, and energy demand (Gerardo et al., 2015). According to Toledo-Cervantes et al., (Toledo-Cervantes et al., 2017a), algal biomass valorization can outweigh the high investment costs of photosynthetic biogas upgrading.

Finally, the European political initiatives for climate change protection and promotion of renewable energies support current growing trend of biomethane

production, with annual estimated values of 1,072 TWh at 57 € MWh^{-1} by 2050, according to the "Gas for Climate: a path to 2050" report (ECOFYS, 2018; Sahota et al., 2018). Moreover, the use of wastes as substrates (biogas from anaerobic digestion and wastewater) together with the production of valuable bioproducts from the algal biomass generated from CO_2 capture during photosynthetic biogas upgrading fosters circular economy and resource efficiency.

4.5   Conclusions

The potential of photosynthetic biogas upgrading coupled with the production of tailored algal biomass has been demonstrated in a Tubular-PBR interconnected to external SC. The influence of alkalinity and N-deprivation in the cultivation broth on biomethane quality and biomass composition was assessed and optimized for 395 days of continuous operation. An alkalinity of ~1700 mg L^{-1} ensured a biomethane composition complying with international regulations for methane injection into natural gas grids or use as vehicle fuel (> 96 % of CH_4). Moreover, the implementation of N feast-famine cycles did not result in a detrimental effect on the biomethane quality, maintaining CO_2-REs of ~97 % and a complete removal of H_2S regardless of the cycle duration. N limitation during 24 and 48 h induced an increase in the carbohydrate content of the harvested biomass up to ~29 % dcw.

Process operation at high alkalinities and N limitation mediated the low microalgae diversity observed in the Tubular-PBR.

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Influence of biogas supply regime on photosynthetic biogas upgrading performance in an enclosed photobioreactor

Influence of biogas supply regime on photosynthetic biogas upgrading performance in an enclosed algal-bacterial photobioreactor

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Highlights

- Biomethane quality was influenced by the biogas supply regime in an enclosed PBR.
- Anoxic H_2S oxidation during the dark period supported continuous biogas upgrading.
- Biogas supply during the dark period increased carbohydrate content by ~24 %.
- Cyanobacteria dominated the culture broth mediated by the high pH and alkalinity.

Graphical abstract
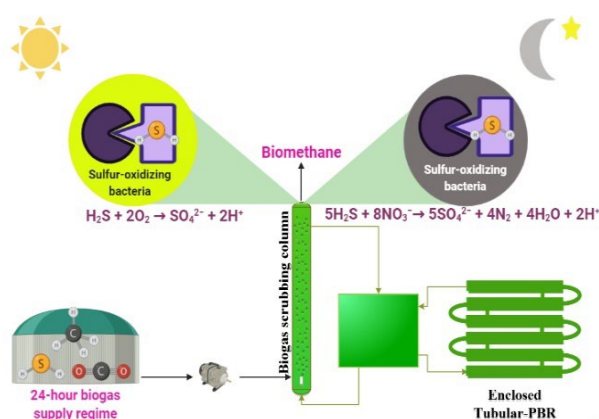
Abstract

Photosynthetic biogas upgrading has recently emerged as an alternative to physical/chemical technologies in order to decrease the energy demand and environmental impact of biomethane production. Despite enclosed photobioreactors (PBRs) provide higher photosynthetic efficiency and CO_2 capture capacity than open ponds, the number of studies of PBRs devoted to biogas upgrading is scarce. The effect of biogas supply regime (12 h feeding during the light period *vs* 24 h feeding during the light and dark periods) on biomethane quality and biochemical biomass composition was evaluated in a 132 L Tubular-PBR interconnected to a 2.5 L biogas scrubbing column operated at high alkalinity. Process operation at a biogas flow rate of 14.4 L d^{-1} during the light period supported a biomethane composition of CH_4 = 95.9 ± 1.3 % v/v, CO_2 = 2.5 ± 0.8 % v/v, N_2 = 1.4 ± 0.6 % v/v, O_2 = 0.1 ± 0.1 % v/v, and negligible H_2S concentrations. On the contrary, CH_4 and CO_2 concentrations of 88.8 ± 1.6 % v/v and 9.3 ± 1.1 % v/v, respectively, were recorded when biogas was continuously supplied (28.8 L d^{-1}). The oxygenation capacity of the Tubular-PBR during the light period was sufficient to oxidize NH_4^+ to NO_3^-, which was further used during the dark period to oxidize H_2S and prevent microalgae inhibition. Biomass productivity and composition remained constant regardless of the biogas supply regime, with a slight increase in carbohydrate content from 23.6 ± 5.7 % (12 h supply) to 30.9 ± 3.5 % (continuous supply).

Keywords: *Alkalinity, Biogas supply regime, Biogas upgrading, Biomethane, Light and dark period, Nitrification-denitrification.*

5.1 Introduction

Fossil fuels accounted for 81 % of the global energy production (14,421 million tonnes of oil equivalent per year), while renewable energy supported a ~14 % in 2018 (IEA, 2020). Most international policies have recently focused on climate change and renewable energy production, with the shared purpose of reducing greenhouse gas emissions and fossil fuel consumption (Sahota et al., 2018). Global investments in renewable energy capacity have reached USD 282 billion in 2019, which represents an increase of 29 % compared with 2010, whereas the installed power capacity reached 2,378 Gigawatts (GW) (REN21, 2020, 2019). In this context, biogas from the anaerobic digestion of organic waste constitutes a source of renewable energy due to its high content of CH_4. The global installed capacity of electricity production from biogas accounted for 19.5 GW in 2019 and the number of anaerobic digesters reached 132,000 (Bose et al., 2020; REN21, 2020).

Raw biogas consists mainly of CH_4 (40 – 70 % v/v), CO_2 (15 – 60 % v/v), H_2S (0.005 – 3 % v/v), N_2 (0 – 2 % v/v), O_2 (0 – 1 % v/v), siloxanes (0 – 0.02 % v/v), volatile organic compounds (VOC, < 0.6 % v/v), NH_3 (< 1 % v/v) and CO (< 0.6 % v/v) (Ryckebosch et al., 2011). The separation of CH_4 from the rest of the components in raw biogas is required if biogas is injected into natural gas grids or used as a transportation fuel (Ángeles et al., 2020b). Technologies such as pressure swing adsorption, water scrubbing, chemical scrubbing, organic solvent scrubbing, membrane separation, and cryogenic separation are nowadays commercially available to convert biogas into biomethane (EBA, 2019; Muñoz et al., 2015). According to the Annual report 2019 of the European Biogas Association (EBA, 2019), the European Union reached a production of 22,744 GW of biomethane in 2018 with 600 plants installed. However, conventional biogas upgrading technologies present disadvantages such as the need for biogas pretreatment, and high energy consumption, operating costs, and environmental impacts (Angelidaki et al., 2019). Indeed, biogas upgrading represents ~30 % of biomethane cost, which nowadays is not competitive with natural gas.

On the other hand, biological biogas upgrading technologies represent a low cost, sustainable, and environmentally friendly alternative to their physical/chemical counterparts. For instance, algal-bacterial photobioreactors (PBRs) interconnected to an external biogas scrubbing column can support a simultaneous removal of CO_2 and H_2S from raw biogas at a low energy demand (ambient temperature and pressure). Photosynthetic biogas upgrading relies on CO_2 fixation by microorganisms (i.e., microalgae or cyanobacteria) via photosynthesis, where the oxygen photosynthetically produced is utilized by sulphur-oxidizing bacteria to oxidize H_2S to SO_4^{2-} (Bahr et al., 2014). The external biogas scrubbing column

supports the gas-liquid mass transport of CO_2 and H_2S to the cultivation broth of the PBR (Angelidaki et al., 2019). This technology has been validated with promising results at pilot and demo scale in open high-rate algae ponds (HRAPs) due to their lower investment cost, simple design, construction, and operation compared to enclosed PBRs (Posten, 2009). This configuration has reached biomethane compositions of 71 – 96 % v/v of CH_4, 0.5 – 17.3 % v/v of CO_2, ~ 0 % v/v of H_2S, 0.4 – 11 % v/v of N_2, and 0 – 6 % v/v of O_2 (Franco-Morgado et al., 2017; Lebrero et al., 2016; Meier et al., 2017; Rodero et al., 2019; Toledo-Cervantes et al., 2017). The use of enclosed PBRs has been proposed as an alternative to open systems in order to prevent excessive N_2 stripping to biomethane and to increase CO_2 fixation capacity during biogas upgrading. However, there is limited knowledge of the performance of this photobioreactor configuration during the dark periods, when photosynthesis stops, H_2S build-up in the cultivation broth might inhibit microalgae growth and CO_2 accumulation could decrease the pH to levels preventing an effective CO_2 capture. In this context, the use of NH_4^+ rich media or wastewater as a nutrient source to support biomass growth could contribute to a beneficial NO_3^- accumulation in the cultivation broth (mediated by NH_4^+ oxidation), which could support anoxic H_2S oxidation during the dark periods and prevent H_2S induced inhibition.

This work investigated the influence of biogas supply regimen (continuous *vs* feeding during the light period) on the photosynthetic biogas upgrading performance of an enclosed Tubular-PBR interconnected to a biogas scrubbing column. In addition, the impact of the biogas supply regime on the production of algal biomass and its biochemical composition was assessed.

5.2   Materials and methods

5.2.1 *Mineral salt medium*

The Tubular-PBR was fed with a modified Brunner medium with the following composition (g L^{-1}): Na_2HPO_4, 2.44; KH_2PO_4, 1.52; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.20; $CaCl_2 \cdot 2H_2O$, 0.05, and 5 mL of SL-4 stock solution. The SL-4 stock solution contained (g L^{-1}): EDTA, 0.5; $FeSO_4 \cdot 7H_2O$, 0.2, and 100 mL of SL-6 stock solution with a composition of (g L^{-1}): $ZnSO_4 \cdot 7H_2O$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.03; H_3BO_3, 0.30; $Co(NO_3)_2 \cdot 6H_2O$, 0.20; $Na_2MoO_4 \cdot 2H_2O$, 0.03; $CuCl_2 \cdot 2H_2O$, 0.01; and $NiCl_2 \cdot 6 H_2O$, 0.02. Chemicals were purchased from Panreac® (Barcelona, Spain) and Sigma-Aldrich® (USA), with a purity of at least 99 %. Inorganic carbon (IC) was added at a concentration of 1600 mg IC L^{-1} using a mixture of 8.0 g $NaHCO_3$ L^{-1} and 3.9 g Na_2CO_3 L^{-1} (Cofarcas S.A., Spain). This mineral salt medium mimicked the concentration of a digestate.

5.2.2 Experimental set-up

The enclosed Tubular-PBR was located indoors at the Institute of Sustainable Processes of Valladolid University. The experimental set-up, with a total working volume of 132 L, consisted of a Tubular-PBR interconnected to a mixing chamber and a biogas scrubbing column (Fig. 5.1). The Tubular-PBR, with 45.6 L of working volume, was composed of 12 transparent tubes of 6 cm of inner diameter and 94 cm of length (total footprint of 0.3 m^2), whereas two sets of high intensity LED PCBs were placed at both sides of the Tubular-PBR to provide a photosynthetic active radiation (PAR) of ~715 µmol m^{-2} s^{-1}. The illumination system was operated under a photoperiod of 12 h light:12 h dark. The cultivation broth was recirculated using a centrifugal pump (DWO/E 300, EBARA, Italy) at a linear velocity of 0.5 m s^{-1} inside the Tubular-PBR. The biogas scrubbing column, with a working volume of 2.5 L (1.73 m height; 4.5 cm internal diameter), was operated co-currently with the cultivation broth from the mixing chamber and synthetic biogas sparged through a stainless-steel diffuser of 2 µm pore size located at the bottom of the column.

5.2.3 Operational procedure

Table 5.1 summarizes the environmental and cultivation broth parameters during photosynthetic biogas upgrading in the enclosed Tubular-PBR. The system was operated for 69 days at 26.8 d of hydraulic retention time, which corresponded to 5 L d^{-1} of cultivation broth exchange rate during the illuminated period. A synthetic biogas mixture composed of CH_4 (70 % v/v), CO_2 (29.5 % v/v) and H_2S (0.5 % v/v) (Abéllo Linde, Spain) was used to evaluate the influence of the raw biogas supply regime on biomethane quality. From days 0 − 34 (stage I), the synthetic biogas was supplied at a flow rate of 14.4 L d^{-1} during the illuminated period (12 h) in the biogas scrubbing column and the cultivation broth was recirculated at a flow rate of 7.2 L d^{-1} (resulting in a L/G ratio of 0.5). In stage II (days 35 − 69), raw biogas was supplied continuously for 24 h per day at 28.8 L d^{-1} at a L/G ratio of 0.5.

5.2.4 Sampling and analytical procedures

5.2.4.1 Gas phase monitoring

Biogas samples (containing CH_4, CO_2, H_2S, N_2, and O_2) were daily drawn from the inlet and outlet of the biogas scrubbing column during the illuminated period and analyzed using a 430 GC-TCD (Bruker, Palo Alto, USA) equipped with a CP-Molsieve 5Å (15 m × 0.53 mm × 15 µm) and a CP-PoraBOND Q (25 m × 0.53 mm × 10 µm) columns. The oven, injector and detector temperatures were maintained at 45, 150, and 200 °C, respectively. Helium was employed as the carrier gas at 13.7 mL min^{-1}. The inlet and outlet flowrates in the biogas scrubbing column were measured to accurately determine CO_2 and H_2S removals efficiencies (REs).

5.2.4.2 Liquid phase monitoring

The temperature, dissolved oxygen (DO) concentration and pH in the cultivation broth were daily measured in the mixing chamber during the illuminated period. The DO concentration and temperature of the cultivation broth were measured with an Oxi 3310 meter connected to a sensor CellOx 325 (WTW, Germany). The pH was measured with a Cyberscan 510 pH meter (Eutech Instruments, The Netherlands). Light intensity as PAR at the Tubular-PBR surface was recorded under each steady-state using a LI−250A light meter (LI-COR Biosciences, Germany). The maximum photochemical yield of photosystem II, represented as the ratio between the maximum variable fluorescence (F_v) and the maximum fluorescence of chlorophyll (F_m) was daily measured in the mixing chamber during the illuminated period using a fluorometer AquaPen AP 110C (Photon Systems Instruments, Drasov, Czech Republic). Liquid samples from the cultivation broth were drawn three times per week from the mixing chamber to analyze the concentrations of IC, total organic carbon (TOC), total nitrogen (TN), ammoniacal nitrogen (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}) and volatile suspended solids (VSS). A TOC-VCSH analyzer (Shimadzu, Japan) equipped with a TNM−1 chemiluminescence-module was used for the determination of IC, TOC, and TN concentrations in filtered samples (0.45 μm). NH_4^+ was measured by an Orion Dual Star specific sensor (ThermoScientific, The Netherlands) in filtered samples (0.45 μm). NO_2^-, NO_3^-, PO_4^{3-}, and SO_4^{2-} analysis was carried out by HPLC-Ion Chromatography in 1.5 mL filtered (0.22 μm) samples according to Posadas et al., (2015b). Biomass concentration as VSS was quantified according to Standard Methods (APHA, 2005).

5.2.4.3 Biochemical and elemental biomass composition analyses

The macroscopic (carbohydrates, lipids, proteins, moisture, and ash content) and elemental (carbon, C; hydrogen, H; nitrogen, N; oxygen, O; and sulphur, S) composition of the biomass were determined under steady-state. The quantitative determination of the carbohydrate content was performed according to the phenol-sulfuric acid method using aliquots of 1.5 mL of biomass harvested from the cultivation broth by centrifugation (1000 rpm, 10 min) (Dubois et al., 1956). The lipid, protein, moisture, and ash content were determined from lyophilized biomass at -80 °C (LyoQuest 55 Plus, Telstar®, Spain). Chloroform in methanol (2:1 v/v) was used for lipid extraction, and the lipid content was calculated gravimetrically according to Kochert, (1978). The protein content was determined by Kjeldahl Total Nitrogen (KTN) and multiplying the result by a factor 5.95 (Owusu-apenten, 2002). The moisture and ash content was quantified

Table 5.1. Average values of the environmental and cultivation broth parameters in the Tubular-PBR under steady state with the corresponding standard deviation (± SD, n = 4 – 30).

Stage	Days	Biogas supply regime (h)	Illumination (h)	DO (mg L^{-1})	T_{PBR} (°C)	F_v/F_m (dimensionless)	TOC (mg L^{-1})	SO_4^{2-} (mg L^{-1})	PO_4^{3-} (mg L^{-1})	NH_4^+ (mg L^{-1})	VSS (mg L^{-1})
I	0 – 34	12	12	15.7 ± 3.3	27.1 ± 1.9	0.40 ± 0.0	130 ± 44	821 ± 132	2505 ± 120	0.0 ± 0.0	513 ± 86
II	35 – 69	24	12	18.3 ± 3.7	28.5 ± 1.5	0.21 ± 0.0	112 ± 56	795 ± 94	2383 ± 150	0.0 ± 0.0	525 ± 66

DO: dissolved oxygen, F_v/F_m: maximum photochemical yield, TN: total nitrogen, TOC: total organic carbon, VSS: volatile suspended solids.

*Biogas was supplied during the illumination period.

according to Van Wychen and Laurens, (2016). The biochemical composition of biomass was expressed as a percentage of dry cell weight (dcw).

The content of C, H, N, O, and S in lyophilized samples was measured using an elemental analyzer (EA Flash 2000, Thermo Scientific TM, USA) equipped with a thermal conductivity detector (TCD). The analysis of C, H, N, and S was carried out at 900 °C with helium as carrier gas at a flowrate of 140 mL min^{-1}, while O was analyzed at 1060 °C and a helium flow rate of 130 mL min^{-1}.

5.2.4.4 Microalgae population structure

The microalgae population structure was quantified through biometry from an aliquot of 1.5 mL of cultivation broth in each steady-state. Samples were fixed with 5 % of lugol acid, and 10 % of formaldehyde (Utermöhl, 1958). Cells were counted and identified according to Sournia, (1978) using an inverted photonic microscope (Nikon IK70, Tokyo, Japan). Finally, taxonomic identification at the genus or species level was determined according to Krammer and Lange-Bertalot, (2000).

5.2.5 Statistical treatment

The time course of biomethane composition, IC, NO_2^-, NO_3^-, and TN concentration, and pH in the cultivation broth was recorded throughout the entire

experiment using duplicate measurements. The temperature, DO, TOC, NH_4^+, PO_4^{3-}, SO_4^{2-}, VSS, the macroscopic and elemental composition of the biomass, and microalgae population structure represent the average under the steady-state operation. CO_2 and H_2S REs were calculated under the steady-state operation based on biogas and biomethane composition. The results here presented were provided as the average values along with their corresponding standard deviation with n = 2 – 30.
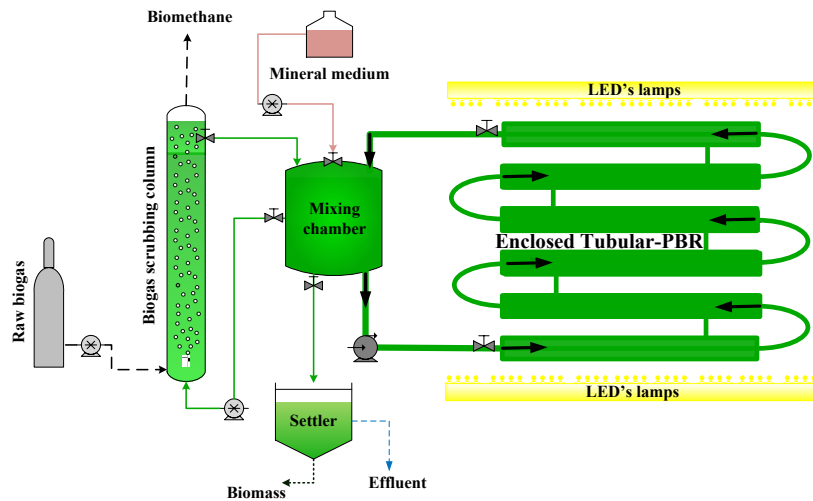

Fig. 5.1 Schematic representation of the experimental set-up of the enclosed Tubular-PBR interconnected to a biogas scrubbing column.

5.3 Results and discussion

5.3.1 *Environmental parameter and operating conditions*

The temperature of the cultivation broth in the Tubular-PBR remained constant at ~27.8 °C during stages I and II, which lies within the optimal range for microalgae growth (15 to 35 °C) (Posadas et al., 2017a). The high photosynthetic activity of microalgae in this PBR supported average DO concentrations of 15.7 ± 3.3 and 18.3 ± 3.7 mg DO L^{-1} in stages I and II, respectively. These high DO values promoted the total oxidation of the ammonium present in the mineral salt medium into nitrate by nitrifying bacteria

(Eq. 5.1), which has been consistently reported in microalgae-based wastewater treatment (Silva et al., 2019).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (Eq. 5.1)$$
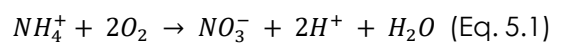
In this context, the high nitrification activity mediated average concentrations of 74.4
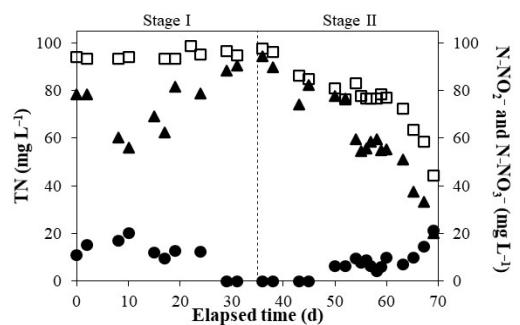
Fig. 5.2. Time course of TN (squares), N-NO$_2^-$ (circles) and N-NO$_3^-$ (triangles) concentrations (mg L^{-1}) throughout stages I and II.

± 11.8 mg N-NO$_3^-$ L^{-1}, 11.0 ± 6.6 mg N-NO$_2^-$ L^{-1}, and 94.7 ± 1.7 mg TN L^{-1} in the cultivation broth during stage I. Nevertheless, when biogas was continuously supplied in stage II, TN concentration in the cultivation broth gradually decreased from 97.5 to 44.4 mg TN L^{-1} (day 69) induced by a concomitant decrease in nitrate concentration from 94.3 mg N-NO$_3^-$ L^{-1} (day 36) to 20.1 mg N-NO$_3^-$ L^{-1} at the end of stage II (Fig. 5.2). Conversely, N-NO$_2^-$ concentration slightly increased up to 20.1 mg N-NO$_2^-$ L^{-1} by day 69. When biogas was supplied during the illuminated period, the O$_2$ photosynthetically produced in the Tubular-PBR was sufficient for sulphur-oxidizing bacteria to oxidize H$_2$S to sulfate (Eq. 5.2), which attained concentrations of 821.0 ± 131.8 mg SO$_4^{2-}$ L^{-1} during stage I. However, when biogas was continuously supplied, sulphur-oxidizing bacteria oxidized H$_2$S during the dark period (when no O$_2$ was *in-situ* produced) using the NO$_3^-$ produced from NH$_4^+$ oxidation during the light period (Eq. 5.3) (Fernández et al., 2014). The accumulation of NO$_2^-$ in the cultivation broth could have been mediated by the partial denitrification of NO$_3^-$ during H$_2$S oxidation (Eq. 5.4). Thus, 8.01 mg of NO$_3^-$ (equivalent to 1.81 mg N) in the cultivation broth are required to oxidize 3 mg of H$_2$S from biogas to sulfate. The concentration of SO$_4^{2-}$ in the cultivation broth during stage II (795.1 ± 94.3 mg SO$_4^{2-}$ L^{-1}) suggested that H$_2$S was also partially oxidized to elemental sulphur (Eq. 5.5, 5.6).

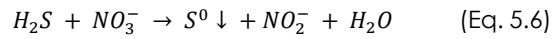$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \qquad \text{(Eq. 5. 2)}$$

$$5H_2S + 8NO_3^- \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O + 2H^+$$

$$\text{(Eq. 5.3)}$$

$$H_2S + 4NO_3^- \rightarrow SO_4^{2-} + 4NO_2^- + 2H^+ \quad \text{Eq. 5.4)}$$

$$H_2S + 0.5O_2 \rightarrow S^0 \downarrow + H_2O \qquad \text{(Eq. 5.5)}$$

$$H_2S + NO_3^- \rightarrow S^0 \downarrow + NO_2^- + H_2O \qquad \text{(Eq. 5.6)}$$
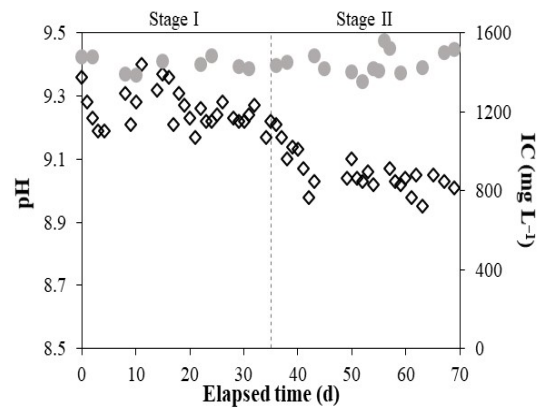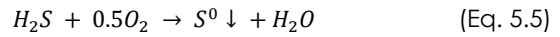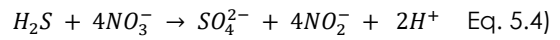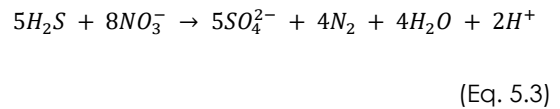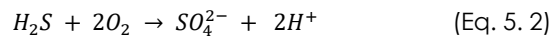

Fig. 5.3. Time course of the pH (diamonds) and inorganic carbon concentration (circles) in the cultivation broth of the Tubular-PBR during stages I and II.

The F$_v$/F$_m$ value, here used as a surrogate of photosynthetic activity, during steady-state I averaged 0.40 ± 0.0, which was similar to the values recorded in a *Tetradesmus wisconsinensis* culture supplemented with IC and CO$_2$, but lower than the F$_v$/F$_m$ value when the same culture was deprived of IC (F$_v$/F$_m$ =0.7) (Janka et al., 2020). Thus, the high IC concentration present in the cultivation broth of the PBR (~1400 mg L^{-1}) could have caused stress in the microalgae culture, since the optimal F$_v$/F$_m$ values of healthy cultures of cyanobacteria and green algae typically range from 0.6 to 0.8

(López-Rosales et al., 2014). Interestingly, the continuous supply of biogas caused significant stress on microalgal metabolisms, as shown by the decrease in F_v/F_m to 0.21 ± 0.0 during stage II. Despite the low photosynthetic efficiency reported, the average VSS concentrations in the cultivation broth remained constant at 513.3 ± 85.7 and 524.6 ± 65.7 mg L^{-1} during stages I and II, respectively.

On the other hand, the pH in the cultivation broth remained stable at 9.3 ± 0.0 when biogas was supplied during the light period (stage I) (Fig. 5.3). This stability was likely mediated by the high IC concentration in the cultivation broth (~1400 mg L^{-1}), which entailed a high buffer capacity. Biogas supply during the light and dark periods decreased the pH to a range of 8.95 – 9.07 during steady-state in stage II, which was due to a CO_2 and H_2S mediated acidification. The negligible CO_2 consumption by microalgae during the dark period induced a swift in the bicarbonate-carbonate equilibrium. The influence of pH on biogas upgrading performance will be discussed in Section 5.3.2.

5.3.2 Biogas upgrading performance

Biomethane composition remained constant during stage I (Fig. 5.4). Thus, average values of CH$_4$, CO$_2$, H$_2$S, N$_2$ and O$_2$ concentration of 95.9 ± 1.3 %, 2.5 ± 0.8 %, 0.0 ± 0.0 %, 1.4 ± 0.6 % and 0.1 ± 0.1 % v/v, respectively, were observed when

biogas was supplied during the light period. This composition fulfilled most international standards for injection into natural gas grids or use as vehicle fuel (CH$_4$ ≥ 95 %, CO$_2$ ≤ 2.5 – 4 %, H$_2$S < 5 mg N m^{-3}, and O$_2$ < 1 %) (Muñoz et al., 2015). Biomethane quality was supported by CO_2 removal efficiencies (REs) of 94.9 ± 2.6 % and complete removals of H_2S (below the detection limit of the GC-TCD ~0.01 g m^{-3}). These high removal efficiencies were mediated by the high IC concentration of ~1400 mg L^{-1} (high buffer capacity) and pH (> 9) of the cultivation broth. A consistent biogas upgrading performance (CO$_2$-REs of 94 – 97 % and a complete H$_2$S removal) has been reported in enclosed and open algal-bacterial photobioreactors interconnected to external biogas scrubbing columns using a highly alkaline solution as the cultivation broth (Ángeles et al., 2020b; Bahr et al., 2014; Posadas et al., 2017b; Rodero et al., 2018a).
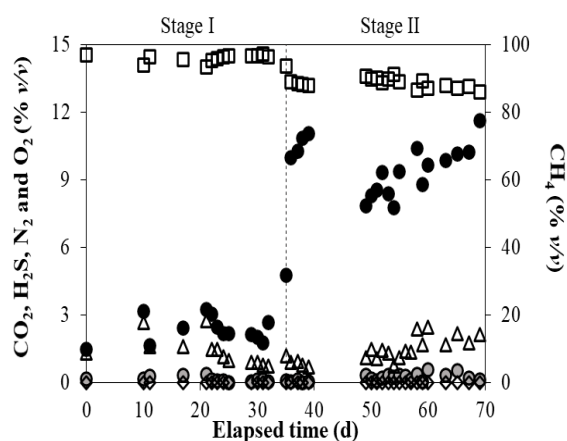

Fig. 5.4. Time course of the biomethane composition during stages I and II: CH$_4$ (squares), CO$_2$ (dark circles), H$_2$S (diamonds), N$_2$ (triangles), and O$_2$ (grey circles).

Nevertheless, biomethane quality decreased when biogas was supplied continuously in stage II (Fig. 5.4). Hence, a steady-state biomethane composition of $CH_4 = 88.8 \pm 1.6$ %, $CO_2 = 9.3 \pm 1.1$ %, $H_2S = 0.0 \pm 0.0$ %, $N_2 = 1.6 \pm 0.5$ % and $O_2 = 0.3 \pm 0.2$ % v/v was recorded as a result of a significant decrease in CO_2-REs to 85.8 ± 4.0 %, whereas H_2S exhibited a complete removal throughout the entire operation of the Tubular-PBR. The increase in biogas loading rate during stage II caused a decrease in the pH of the cultivation broth that negatively affected the final biomethane quality (Fig. 5.3 and 5.4), despite culture broth alkalinity remained stable throughout the entire experiment. In this context, Ángeles et al., (2020a) observed CO_2 contents < 2 % v/v when the pH was maintained > 9.3 during photosynthetic biogas upgrading in an enclosed system operated at 1300 – 1700 mg IC L^{-1} with biogas supply during the light period. Therefore, our results confirmed that slight variations in the pH of the cultivation broth significantly influenced the mass transfer rates of the acid gases CO_2 and H_2S, which ultimately impacted on the final biomethane quality (Bahr et al., 2014; Posadas et al., 2017b). The higher H_2S removals compared to those recorded for CO_2 were mediated by its higher aqueous solubility (more than three times higher than that of CO_2) (Sander, 2015). Finally, the content of N_2 and O_2 in the biomethane remained below the limits of most international standards for injection into natural grids or use as vehicle fuel regardless of the biogas supply regime. Hence, the low L/G ratio of 0.5 implemented during the operation of the Tubular-PBR supported low desorption of N_2 and O_2 from the cultivation broth to the upgraded biogas.

5.3.3 *Effect of biogas supply regime on biomass productivity, composition, and structure*

The areal biomass productivity in the Tubular-PBR during steady-state was calculated according to the PBR footprint (Posten, 2009). A negligible impact of the biogas supply regime was observed on biomass productivity, which reached values of 9.7 ± 1.6 and 9.9 ± 1.2 g m^{-2} d^{-1} in stages I and II, respectively (Table 5.2). Areal biomass productivities ranging from 2.2 to 9.7 g m^{-2} d^{-1} have been reported in indoor HRAPs devoted to photosynthetic biogas upgrading with biogas supplied during the light period (Franco-Morgado et al., 2017; Rodero et al., 2018a; Alma Toledo-Cervantes et al., 2016). On the contrary, a decrease in biomass productivity of ~80 % was observed when biogas was continuously supplied during the light and dark periods as a result of H_2S inhibition in a HRAP (Franco-Morgado et al., 2017).

On the other hand, a slight increase in carbohydrate content from 23.6 ± 5.7 % to 30.9 ± 3.5 % dcw was recorded when biogas was also supplied during the dark period. Similar values of carbohydrates content have been obtained in diverse

genera of microalgae and cyanobacteria such as *Chlorococcum*, *Tetraselmis* and *Spirulina* (Martínez-Roldán and Ibarra-Berumen, 2019). The lipid content remained constant at ~8 % dcw regardless of the biogas supply regime, which lied within the typical ranges of lipid contents during photosynthetic biogas upgrading (2.9 to 17 % dcw) (Posadas et al., 2016) but below the optimal ranges for valorization as biodiesel feedstock (20 – 70 % dcw) (Chisti, 2007; Koutra et al., 2018). The protein content of the algal-bacterial biomass during stages I and II was 48.2 ± 1.4 and 52.9 ± 1.8 % dcw, respectively, slightly higher than the average protein content of ~40 % previously observed during photosynthetic biogas upgrading in HRAPs (Posadas et al., 2016, 2017b; A Toledo-Cervantes et al., 2016). In this context, an effective valorization of the algal biomass produced during CO_2 fixation could contribute to create new value chains in the biogas sector and increase the economic sustainability of the process. For instance, a selling price of 0.23 € kg dcw⁻¹ and 0.06 € kg dcw⁻¹ of whole microalgae biomass could be targeted for bioethanol or biodiesel production, according to Bose et al., (2020). Moreover, a sales price of 0.08 € kg dcw⁻¹ of algal biomass as biofertilizer could be attained based on its high content of proteins (Ecocelta Galicia S.L.). Carbon was the most abundant element in microalgae biomass with shares of 42.4 ± 0.6 % and 44.2 ± 0.2 % dcw during stages I and II, respectively (Table 5.2). Similarly, H and O contents remained constant at

Table 5.2. Table 2. Influence of biogas supply regime on the areal biomass productivity, biochemical and elemental composition of the biomass. Values are presented as the steady-state average with the corresponding standard deviation (± SD, n = 2 – 12).

Stage	Areal biomass productivity (g m⁻² d⁻¹)	Chemical composition (% dcw)					Elemental composition (% dcw)				
		Carbohydrates	Lipids	Proteins	Ashes	Moisture	C	H	N	O	S
I	9.7 ± 1.6	23.6 ± 5.7	8.8 ± 2.8	48.2± 1.4	15.4 ± 1.7	4.8 ± 1.7	42.4 ± 0.6	6.8 ± 0.2	7.8 ± 0.1	34.5 ± 1.7	0.6 ± 0.0
II	9.9 ± 1.2	30.9 ± 3.5	8.8 ± 2.7	52.9 ± 1.8	8.4 ± 1.3	1.3 ± 0.2	44.2 ± 0.2	7.1 ± 0.1	8.7 ± 0.1	32.1 ±0.2	0.7 ± 0.0

~6.9 and ~33 % dcw, respectively. On the other hand, the N and S content slightly increased from 7.8 ± 0.1 to 8.7 ± 0.1 %, and from 0.6 ± 0.0 % to 0.7 ± 0.0 % dcw, respectively, when the biogas was continuously supplied in the light and dark periods. These values match the elemental compositions of the algal bacterial biomass previously reported during photosynthetic biogas upgrading: C (47 – 32 % dcw), N (8 – 6 % dcw), and S (0.7 – 0.2 % dcw) (Posadas et al., 2016, 2017b; Rodero et al., 2019; A Toledo-Cervantes et al., 2016).

Finally, the cyanobacterium *Pseudanabaena* sp. was dominant (58 %) at the end of stage I, followed by the green microalgae *Chlorella vulgaris* with a share of 29 % (Fig. 5.5). This cyanobacterium is a common species in freshwater, although it has been recently found during photosynthetic biogas upgrading in open systems (Marín et al., 2018; Posadas et al., 2017b, 2015b). At the end of stage II, *Pseudanabaena* sp. accounted for 95 % of the total number of algal cells. The dominance of cyanobacteria over green algae species is typically promoted by some factors such as nitrogen deficiency and high inorganic carbon concentrations and pH values in the cultivation broth (Ángeles et al., 2020a; Arias et al., 2017; Troschl et al., 2018). On the other hand, *Tetracoccus* sp., a H_2S oxidizing-bacteria, was found in stages I and II with abundances of up to 12%.
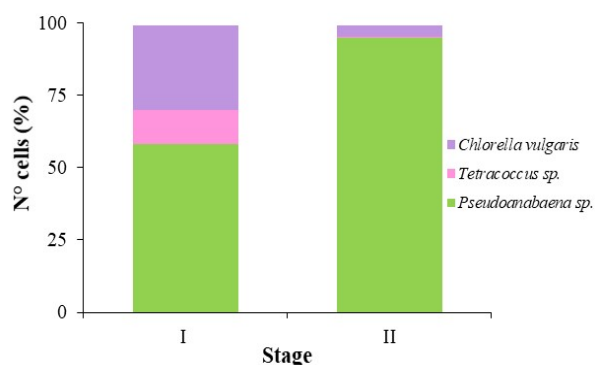
Fig. 5.5 Microalgae and bacteria population structure in the enclosed Tubular-PBR during stages I and II.

5.4 Conclusions

This study confirmed the feasibility of continuous biogas upgrading in an enclosed Tubular-PBR as a result of the effective H_2S oxidation during the dark period using the NO_3^- produced from NH_4^+ oxidation during the light period. This prevented microalgae inhibition induced by H_2S build-up in the cultivation broth. The high alkalinity in the cultivation broth and the low operational L/G ratios in the biogas scrubbing column supported a complete elimination of H_2S and limited desorption of N_2 and O_2. However, lower CO_2 removals were recorded as a result of the acidification of the cultivation broth (pH ≤ 9) when raw biogas was supplied continuously for 24 h per day. Biomass productivity remained constant under the two different biogas supply regimes, while carbohydrate content increased by ~24 % under continuous biogas supply. Interestingly, a low microalgae diversity, with a dominance of *Pseudanabaena* sp., was recorded throughout the entire operation of the PBR, which was

attributed to the high alkalinity and pH prevailing in the culture broth.

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Biogas-based production of glycogen by *Nostoc muscorum:* Assessing the potential of transforming CO₂ into value added products

Biogas-based production of glycogen by *Nostoc muscorum*: assessing the potential of transforming CO_2 into value added products

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Highlights

- *Nostoc muscorum* was able to grow under CO_2 concentrations up to 30 % *v/v*.

- Biogas was a feasible low-cost feedstock for glycogen accumulation in *N. muscorum*.

- Valeric acid enhanced glycogen accumulation to 60 % dcw under nutrient deprivation.

- Negligible PHB synthesis was recorded in *N. muscorum* when cultivated at 30% *v/v* CO_2.

Graphical abstract
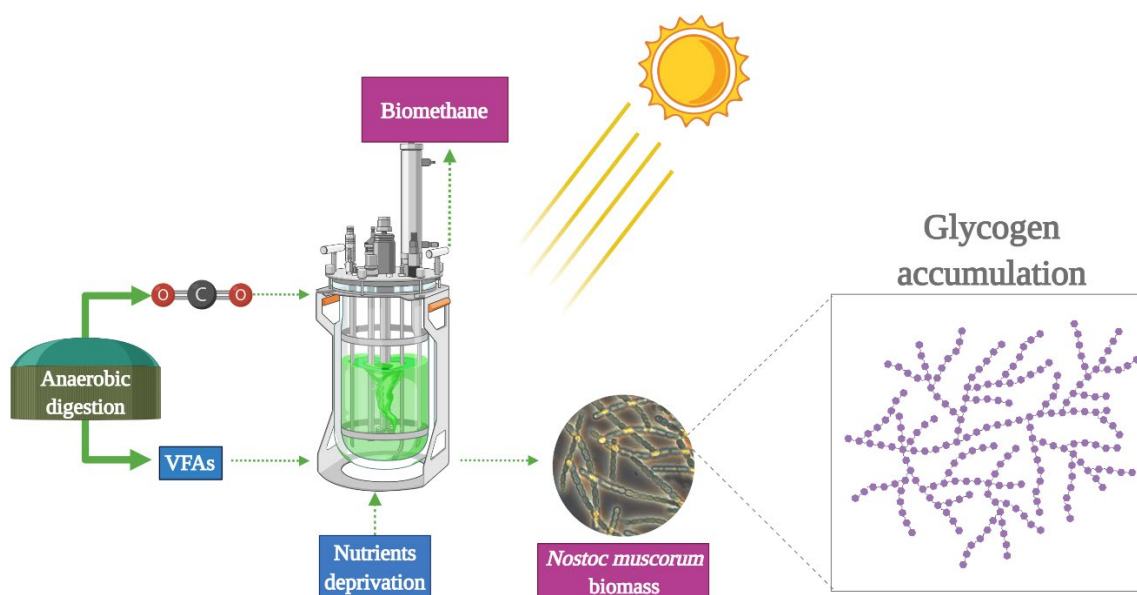
Abstract

The potential of the filamentous N_2-fixing cyanobacterium *Nostoc muscorum* for CO_2 capture from high-loaded streams (i.e. flue gas or biogas) combined with the accumulation of glycogen (GL) and polyhydroxybutyrate (PHB), was evaluated under nutrient-sufficient and nutrient-limited conditions. *N. muscorum* was able to grow under CO_2 contents from 0.03 up to 30 % *v/v*, thus tolerating CO_2 concentrations similar to those found in raw biogas or flue-gas, with maximum CO_2-fixation rates of 191.9 ± 46 g m^{-3} d^{-1} at a biomass concentration of 733.3 ± 207.4 mg TSS L^{-1}. Despite *N. muscorum* was inhibited by the presence of H_2S, the co-inoculation with activated sludge resulted in both CO_2 and H_2S depletion. Moreover, *N. muscorum* accumulated GL up to ~54 % dcw under N and P-deprivation, almost 36 times higher than that recorded under nutrients sufficient condition. The addition of 10 % extra carbon in the form of valeric acid not only did not hamper the growth of *N. muscorum* (336.0 ± 113.1 mg TSS L^{-1}) but also increased the GL content to ~58 % dcw. On the contrary, a negligible PHB accumulation was found under the tested conditions, likely due to the high CO_2 concentration of 30 % *v/v* in the headspace and therefore the high availability of inorganic carbon for the cultures. *N. muscorum* cultures achieved VFAs degradations up to ~78 % under controlled pH. These results supported *N. muscorum* as a sustainable alternative for CO_2-capture and greenhouse gas mitigation or for photosynthetic biogas upgrading coupled with value added biomass production.

Keywords: *CO_2 fixation, Glycogen accumulation, Nostoc muscorum, Nutrient deprivation, Poly–β–hydroxybutyrate accumulation*

6.1 Introduction

Carbon dioxide (CO_2) is the most important greenhouse gas (GHG), with a share of approximately 78 % of the total GHG emissions worldwide and an annual atmospheric concentration increase of 0.5 % over the last decade (IPCC, 2014). Fossil fuel combustion and industrial processes represent ~60 % of the global CO_2 emissions to the atmosphere (Mustafa et al., 2020). Current technologies for CO_2 capture such as absorption, adsorption or membranes, are limited by their high energy consumption and the associated costs, which can amount to 138 € per ton of CO_2 captured (Terlouw et al., 2019; Wilberforce et al., 2021). Furthermore, operational limitations such as corrosion, large requirements of water, or the stability of chemical solvents for CO_2 absorption are important disadvantages of physical-chemical technologies (Al-Mamoori et al., 2017). Additionally, commercially available adsorbents offer low CO_2 selectivity and adsorption capacity, resulting in lower removal efficiencies compared to other physical-chemical counterparts, together with the difficult regeneration and reusability of adsorbents (Song et al., 2019). Moreover, membranes are very sensitive to temperature (which cannot exceed 100 ºC) and to corrosive gases such as SO_x, NO_x, and H_2S, limiting their performance over the long-term operation (Song et al., 2019). Therefore, recent trends towards sustainability focus on environmentally friendly and cost-effective technologies such as the fixation of CO_2 by microalgae and cyanobacteria via photosynthesis, which has emerged as a sustainable and economic alternative for CO_2 abatement and biomass production. The potential for CO_2 fixation has been investigated for several photosynthetic microorganisms such as *Chlorella* sp., *Scenedesmus obliquus*, or *N. muscorum* (Bhati and Mallick, 2016; Li et al., 2020; Sydney et al., 2019).

Cyanobacteria (gram-negative prokaryotes) are photosynthetic microorganisms able to fix CO_2 via the Calvin-Benson-Bassham (CBB) cycle (Fig. 6.1) (Venkata Mohan et al., 2016). This primary carbon fixation pathway consists of 13 reactions and involves 11 enzymes, ribulose–1,5–bisphosphate carboxylase/oxygenase (RuBisCO) being the main CO_2-fixing enzyme. In the CBB cycle, light energy is first used during photosynthesis to produce adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH), which are the precursors that drive the synthesis of carbohydrate molecules (i.e. glyceraldehyde–3–phosphate) (Durall and Lindblad, 2015; Liang and Lindblad, 2016). Besides fixing CO_2, many cyanobacteria have the ability to fix atmospheric N_2 while carrying out oxygenic photosynthesis, and require some simple inorganic nutrients to grow using solar energy (Balaji et al., 2013; Jacobsen and Frigaard, 2014). Moreover,

cyanobacteria can synthesize and accumulate more than 1100 secondary metabolites, hence, the versatility of cyanobacterial biomass for biotechnological applications has been extensively investigated. For instance, microalgae biomass can be valorized into bioactive compounds (i.e. proteins, fatty acids, polysaccharides, enzymes, vitamins, sterols and several other high-value compounds having pharmaceutical and nutritional applicability), for the production of biofertilizers and microalgal biofules, or even used in environmental detoxification (Abed et al., 2009; Goleman et al., 2017; Kumar et al., 2018). Among these metabolites, glycogen (GL) and poly–β–hydroxybutyrate (PHB) are value added compounds produced by cyanobacteria using CO_2 as the sole carbon source via the CBB cycle (Fig. 6.1) (Kamravamanesh et al., 2018).

GL is a multibranched polysaccharide of glucose that serves as a form of energy storage in some microorganisms such as cyanobacteria, which can accumulate up to 70 % dry cell weight (dcw). Due to its physicochemical properties, GL is commonly applied in the pharmaceutical industry as a drug delivery vehicle (Vidal and Venegas-Calerón, 2019). On the other hand, PHB is the most common polyhydroxyalkanoate (PHA) synthesized by prokaryotic microorganisms, able to accumulate up to 55 % dcw under photoautotrophic growth and nutrient starvation (e.g., N and P limitation) (Haase

et al., 2012; Samantaray and Mallick, 2012; Shrivastav et al., 2010). PHB is regarded as a "green thermoplastic" since it is a biologically originated, degradable and renewable substance with similar properties to those of petroleum-based plastics (Ángeles et al., 2019). Several studies have evidenced the ability of cyanobacteria to accumulate intracellular GL and/or PHB under nutrient deprivation conditions (N and P) (Bhati and Mallick, 2016; Rueda et al., 2020), although some strains also require the supplementation of an organic carbon substrate to promote biopolymer production. According to Bhati and Mallick (2015), *N. muscorum* is one of the photosynthetic microorganisms with the highest accumulation potential of the P(3HB–co–3HV) copolymer, reaching values up to 78 % dcw under N-deprivation when supplemented with 0.28 % (w/v) acetate, 0.38 % (w/v) glucose, and 0.3 % (w/v) valerate.

Additionally, the use of a low-cost carbon source for GL and PHB accumulation in photosynthetic microorganisms will allow for a significant reduction of the production costs, as it usually accounts for 30 – 40 % of the total costs (Rodríguez et al., 2020). In this context, CO_2 represents an undesired component raw biogas from anaerobic digestion, reaching concentrations up to 30 % v/v. Its removal is of key interest to reduce transportation costs and greenhouse gas emissions and to increase the biogas specific energy content. Thus, photosynthetic biogas

upgrading by cyanobacteria coupled with the production of high-value biomass constitutes a sustainable and environmentally friendly alternative to conventional physical-chemical methods that could boost the cost-effectiveness of CO_2 capture.

This study aimed at optimizing both the growth and the accumulation of value added products (i.e. GL and PHB) by the cyanobacterium *N. muscorum* using CO_2 from either biogas or flue-gas as the carbon source in laboratory-scale experiments. Different operating parameters were studied and optimized the composition of the gas headspace, the C:N and C:P ratios, and the use of VFAs (acetic and valeric acids) in combination with CO_2 fixation.
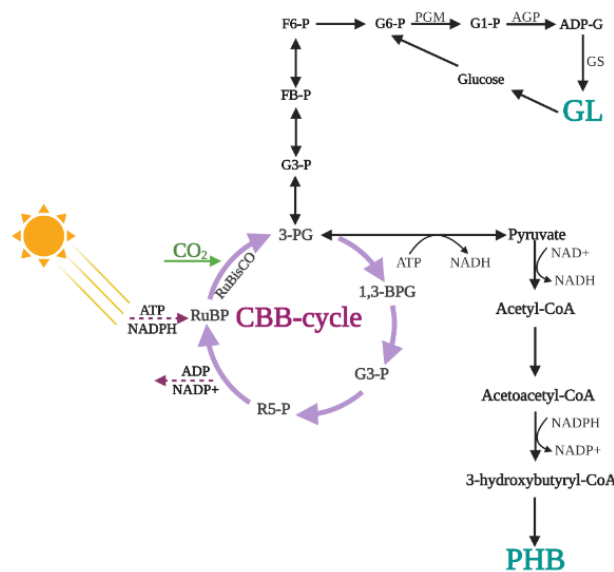
Fig. 6.1. Metabolic pathway for carbon assimilation and production of glycogen (GL) and polyhydroxybutyrate (PHB) by cyanobacteria through the Calvin-Benson-Bassham cycle (CBB): ADP, adenosine–diphosphate; ADP–G, ADP–glucose pyrophosphorylase; AGP, adenosine diphosphate glucose pyrophosphorylase; ATP, adenosine-triphosphate; FB–P, fructose–1,6–bisphosphate; F6–P, fructose 6–phosphate; GS, glycogen synthase; G1–P, glucose–1–phosphate; G3–P, glyceraldehyde–3–phosphate; G6–P, glucose–6–phosphate; NADH, nicotinamide adenine dinucleotide (reduced); NAD$^+$, nicotinamide adenine dinucleotide (oxidized); NADPH, nicotinamide adenine dinucleotide phosphate (reduced); NADP$^+$, nicotinamide adenine dinucleotide phosphate (oxidized); PGM, phosphoglucomutase; RuBisCO, ribulose–1,5–biphosphate carboxylase/oxygenase; RuBP, ribulose–1,5–bisphosphate; R5–P, ribulose 5–phosphate; 3–PG, 3–phosphoglycerate. (Adapted from Monshupanee and Incharoensakdi, (2014); Venkata Mohan et al., (2016). Figure created with BioRender.com).

6.2 Materials and methods

6.2.1 *Inoculum and basal medium*

The filamentous N_2-fixing cyanobacterium, *N. muscorum* (1453–12b) was obtained from the culture collection of algae (SAG) at the University of Göttingen (Göttingen, Germany). It was grown under sterile conditions in 250 mL flasks containing 100 mL modified Esddekok+Salze (ES) medium (SAG, 2008). The medium constituents (g L^{-1}) were KNO_3, 1.2; K_2HPO_4, 0.02; $MgSO_4·7H_2O$, 0.02; and 5 mL of micronutrient solution. The micronutrient solution contained (g L^{-1}) $ZnSO_4·7H_2O$, 0.001; $MnCl_2·4H_2O$, 0.002; H_3BO_3, 0.01; $Co(NO_3)_2·6H_2O$, 0.001; $Na_2MoO_4·2H_2O$, 0.001; $CuSO_4·5H_2O$, 0.000005; $FeSO_4·7H_2O$, 0.7, and EDTA (disodium magnesium salt), 0.8.

6.2.2 *Chemicals*

All chemicals were purchased from Panreac® (Barcelona, Spain) with a purity of at least 99 %. Synthetic biogas mixture (70 % CH_4, 29.5 % CO_2, 0.5 % H_2S) and synthetic flue-gas (30 % CO_2, 70 % N_2) were acquired from Abelló Linde S.A. (Barcelona, Spain). Poly[(R)–3–hydroxybutyric acid co–(R)–3–hydroxyvaleric acid] (molar ratio 88/12, ≥ 99.99 %) and valeric acid (≥ 99 %) were obtained from Sigma-Aldrich® (USA). Acetic acid (≥ 99 %) was purchased from Cofarcas S.A. (Burgos, Spain).

6.2.3 *Experimental set up and operating procedure*

The experimental set-up consisted of 2.2 L serum bottles capped with butyl-rubber stoppers and aluminum crimp seals (Fig. 6.2). The bottles were maintained under continuous agitation using magnetic stirrers (Poly 15 Variomag, Thermo Scientific ™, Langenselbold, Germany) at 300 rpm and an illuminated area of 0.01 m^2. Photosynthetic active radiation (PAR) of 275.5 ± 24.9 µmol m^{-2} s^{-1} was provided using two high intensity LED PCBs (Phillips SA, Spain), and a photoperiod of 12 h light and 12 h dark cycles was set. All Test Series were carried out in duplicate in a temperature-controlled room at 24.9 ± 1.4 °C.

For inoculum preparation, the cultures were grown in 250 mL flasks containing 100 mL ES medium and continuously stirred at 150 rpm. Once the culture reached the exponential growth phase, the biomass was harvested from the cultivation flasks by centrifugation (10000 rpm, 10 min) and resuspended in 2.2 L serum bottles containing 0.66 L of fresh ES medium (Test Series 1 and 2) or ES medium with low N content (~10 mg N L^{-1}) (Test Series 3, 4 and 5). In the latter case, a second growth stage was performed in the N-deprived medium prior to Test Series 3-5 (Fig. 6.2). The biomass was harvested by centrifugation (10000 rpm, 10 min) once the culture reached the exponential growth phase and resuspended in the corresponding mineral medium (ES, ES N-deprived, or ES P-deprived depending on

the batch Test Series). All conditions were tested in duplicate.

Gas mixtures were prepared in 2 L Tedlar® gas sampling bags (Sigma-Aldrich®, USA) injecting the appropriate volumes of synthetic flue gas or biogas from the cylinders (see Test Series description). The content of the bag was pumped using an ELECTRO A.D C5 diaphragm vacuum pump (ELECTRO A.D, S.L., Barcelona, Spain) through a filter to prevent contamination (0.22 μm, fiberglass) into the corresponding bottle in order to completely flush the air atmosphere out.

6.2.3.1 Test Series 1: Influence of CO_2 concentration on N. muscorum growth

The influence of the composition of the flue gas on *N. muscorum* growth was evaluated under five different CO_2-supplemented atmospheres: 0.03, 5, 10, 20, and 30 % v/v. The 2.2 L serum bottles contained ES medium with 161.9 ± 3.0 mg $N-NO_3^-$ L^{-1} at a pH of 7.1 ± 0.8 and were inoculated at an initial biomass concentration of 108.6 ± 0.0 mg TSS L^{-1}.

6.2.3.2 Test Series 2: Influence of H_2S-loaded biogas on N. muscorum growth

The ability of *N. muscorum* to grow on biogas with H_2S was assessed under a synthetic biogas mixture (CH_4 70 %, CO_2 29.5 %, H_2S 0.5 % v/v). A control bottle

using H_2S-free biogas (CO_2 30 % and N_2 70 % v/v) and an abiotic control to rule out any potential CO_2 removal due to adsorption or photolysis were also prepared as above described. The bottles contained ES medium with 177.6 ± 2.6 mg $N-NO_3^-$ L^{-1} at a pH of 5.8 ± 0.1 and were inoculated at an initial biomass concentration of 353.3 ± 49.3 mg TSS L^{-1} (except for the abiotic control). Two of the biogas-loaded bottles were also inoculated with 5 mL of activated sludge from the Valladolid wastewater treatment plant at a concentration of 10130 mg TSS L^{-1}.

6.2.3.3 Test Series 3: Accumulation of value added compounds under N and P deprivation

The potential for GL and PHB accumulation was analyzed under N-deprivation (10.8 ± 0.9 mg $N-NO_3^-$ L^{-1}) at different P concentrations: 2.5 ± 0.0, 1.1 ± 0.0 and 0.2 ± 0.1 mg $P-PO_4^{3-}$ L^{-1}, corresponding to N:P ratios of ~4, 10 and 54. A control bottle with N concentration of 151.6 ± 4.8 mg $N-NO_3^-$ L^{-1} was included. A flue gas mixture composed of CO_2 and N_2 (30 and 70 % v/v, respectively) was supplemented to the bottles' headspace, which were then inoculated at an initial biomass concentration of 109.0 ± 17.6 mg TSS L^{-1}. By day 1.8, once the CO_2 in the headspace was depleted, 1.2 mL of a HCl solution (3 M) were added in order to

desorb the remaining CO_2 from the cultivation broth.

6.2.3.4 Test Series 4: Influence of VFAs on the accumulation of value added compounds under N deprivation

The influence of acetic and valeric acids on GL and PHB accumulation was evaluated under both N-excess (172.5 ± 5.8 mg $N-NO_3^-$ L^{-1}) and N-deprivation (12.5 ± 2.4 mg $N-NO_3^-$ L^{-1}). Prior inoculation, the bottles were supplemented with a flue gas mixture (30 % CO_2 and 70 % N_2 v/v), and the pH of the ES medium was adjusted to ~7.3 ± 0.1 by addition of NaOH solution (3 M). The bottles were inoculated at an initial biomass concentration of 91.2 ± 22.1 mg TSS L^{-1}. Once the CO_2 was depleted by day 3.3, 10 % v/v of the C initially supplied as CO_2 was added as acetic or valeric acid depending on the condition evaluated. No VFA was supplemented to the control bottles.

6.2.3.5 Test Series 5: Influence of VFAs on the accumulation of value added compounds under N and P deprivation

The influence of acetic and valeric acids on the accumulation of GL and PHB was evaluated under both N and P excess (162.1 ± 2.7 mg $N-NO_3^-$ L^{-1} and 3.6 ± 0.0 mg $P-PO_4^{3-}$ L^{-1}), and N and P deprivation (2.2 ± 2.3 mg $N-NO_3^-$ L^{-1} and 0.0 ± 0.0 mg $P-PO_4^{3-}$

L^{-1}). Prior inoculation, the bottles were supplemented with a flue gas mixture (30 % CO_2 and 70 % N_2 v/v), and the pH of the ES medium was adjusted to ~7.5 ± 0.0 by addition of NaOH solution (3 M). Moreover, 10 % v/v of the C supplied as CO_2 was also added as acetic or valeric acid depending on the condition evaluated, no VFA being supplemented to the control bottles. The initial biomass concentration was 187.2 ± 78.3 mg TSS L^{-1}.

The composition of the gas headspace (CH_4, CO_2, and H_2S) was periodically measured in all bottles. Likewise, 25 mL samples from the cultivation broth were withdrawn at the beginning and the end of each Test Series for the determination of pH and TSS, TN, NO_2^-, NO_3^-, PO_4^{3-} and VFAs concentration when applicable. In Test Series 3, additional samples were withdrawn by day 1.8 analysis (prior HCl addition). GL and PHB accumulation were also quantified from biomass samples at the beginning and the end of Test Series 3, 4, and 5.

6.2.4 Analytical methods

CH_4, CO_2, and H_2S concentrations in the gas headspace were analyzed by gas chromatography in a Bruker 430 GC-TCD (Bruker, Palo Alto, USA) equipped with a CP-Molsieve 5Å (15 m × 0.53 mm × 15 µm) and a CP-PoraBOND Q (25 m × 0.53 mm × 10 µm) columns. The oven, injector, and detector temperatures were maintained at 45, 150, and 200 °C, respectively. Helium was used as the carrier gas at

13.7 mL min^{-1}. The determination of TSS concentration (mg L^{-1}) was carried out according to standard methods (APHA, 2005), using 20 mL of filtered samples (0.45 μm) from the cultivation broth. The CO_2 fixation potential was calculated by equation 6.1 (Razzak et al., 2017), where R_{CO_2} is the rate of CO_2 fixation (g m^{-3} d^{-1}), P is the biomass concentration as TSS (mg L^{-1}), C_{CO_2} is the carbon content of cyanobacterial biomass obtained from CO_2, and M_{CO_2} and M_C are the molecular weights of carbon dioxide and carbon, respectively.

$$R_{CO_2} = P \cdot C_{CO_2} \left(\frac{M_{CO_2}}{M_C} \right) \qquad \text{(Eq. 6.1)}$$

The pH was measured with a pH-Meter Basic 20$^+$ (Crison Instruments, S.A., Barcelona, Spain). Total nitrogen (TN) concentration was determined in the cultivation broth following sample filtration (0.45 μm) using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module, while the concentration of NO_2^-, NO_3^- and PO_4^{3-} was measured by HPLC-IC in 1.5 mL of filtered samples (0.22 μm, fiberglass) through a Waters 515 HPLC pump coupled with an Ion conductivity detector (Waters 432), and equipped with an IC-PACK Anion HC column (4.6 mm × 150 mm) and an IC-PAK Anion Guard-Pak (Waters). Samples of 1.5 mL of the cultivation broth were filtered (0.22 μm, fiberglass) and acidified with 20 μL H_2SO_4 (96 – 97 % w/v) for the determination of VFAs concentration. The analysis was performed in an Agilent 7820A GCFID

(Agilent Technologies, Santa Clara, USA) equipped with a G4513A autosampler and a TEKNOKROMQ NF 29370-F packed column (2 m × 1/8" × 2.1 mm) (Teknokroma, Barcelona, Spain). The injector, oven and detector temperatures were maintained at 300, 135, and 350 °C, respectively. N_2 was used as the carrier gas at 10 mL min^{-1}.

For GL extraction, 1.5 mL of the cultivation broth were centrifuged (10000 rpm, 10 min) and the pellets were resuspended in 100 μL of deionized water on an ice bath. The samples were then homogenized and boiled for 5 min to inactivate the enzymes, cooled in an ice bath, and centrifuged (10000 rpm, 5 min) to remove insoluble material before extracting the supernatant. GL content was determinate by enzymatic hydrolysis using a *Glycogen Assay Kit* MAK016 (Sigma-Aldrich®, USA) and a Fluorimeter FLUOstar® Optima (BMG LABTECH, Germany) to measure the fluorescence intensity (λ_{ex}=540/λ_{em}=587 nm). A blank of ES medium was included in each reaction and subtracted from the sample readings. GL concentration was estimated from the standard curve using the GL standard (2.0 mg mL^{-1}). GL content was expressed as percentage of dry cell weight (% dcw).

Quantitative determination of PHB was carried out by adapting the method described by Zúñiga et al., (2011). Samples of 1.5 mL were centrifuged (10000 rpm, 10 min) and the supernatant was discarded. Then, 1mL of a solution of 1–propanol:HCl (80:20 *v/v*, 37 % HCl

(*w/v*)), 50 μL of internal standard (benzoic acid in 1–propanol (25 g L⁻¹)) and 2 mL of chloroform were added to the pellets and incubated for 4 h at 100 °C in a Thermoreaktot TR 300 (Merck KGaA, Darmstadt, Germany). After digestion, 1 mL of deionized water was added, and the suspension was vortexed. The organic phase was collected and filtered through 0.22 μm fiberglass filters. The PHB content was measured in a 7820A GC coupled with a 5977E MSD (Agilent Technologies, Santa Clara, USA) and equipped with a

DB-wax column (30 m × 250 μm × 0.25 μm). The detector and injector temperatures were maintained at 250 °C. The oven temperature was initially maintained at 40 °C for 5 min, increased at 10 °C min⁻¹ up to 200 °C, maintained at this temperature for 2 min and then increased up to 240 °C at a rate of 5 °C min⁻¹. Finally, area values were expressed as percentage dry cell weight based on the standard curve of Poly[(R)–3–hydroxybutyric acid *co*–(R)–3–hydroxyvaleric acid] (Sigma-Aldrich®, USA).
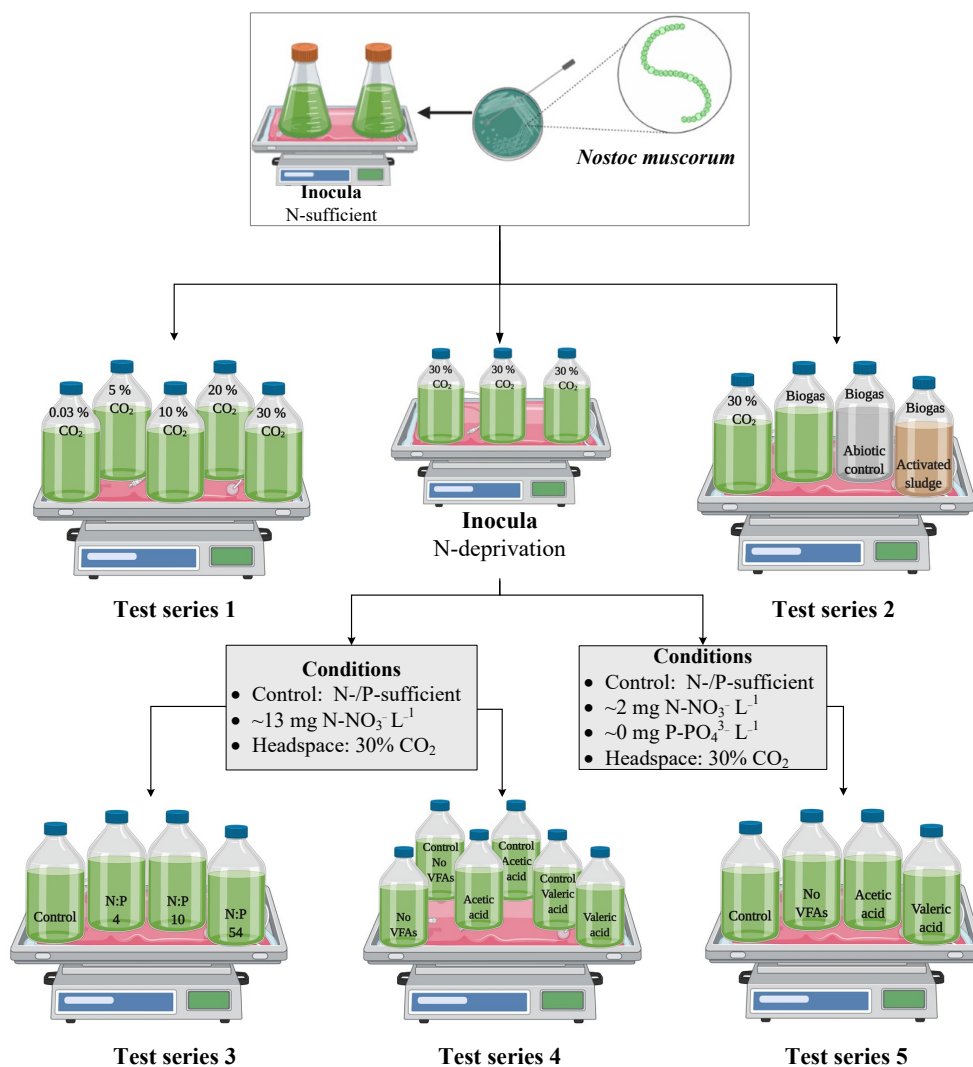
Fig. 6.2. Experimental conditions of the different Test Series (Figure created with BioRender.com).

6.3   Results and discussion

6.3.1 *Influence of CO_2 concentration and H_2S presence on N. muscorum growth*

No detrimental effect on the growth of *N. muscorum* was observed even at the highest CO_2 concentration tested (30 % *v/v*), which demonstrated the tolerance of this cyanobacterium to high CO_2-loaded atmospheres (Fig. 6.3). In this context, few photosynthetic microorganisms such as *Scenedesmus obliquus*, *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Spirulina platensis*, *Anabaena* sp. or *Euglena graciliis* are capable of growing at CO_2 concentrations > 20 % *v/v* (Arbib et al., 2014; Bose et al., 2019; Tang et al., 2011). Interestingly, *N. muscorum* also showed the capacity to grow under a highly CO_2 concentrated atmosphere (30 % *v/v)* with no evidence of inhibition. Hence, raw biogas from the anaerobic digestion of solid waste or flue-gas, with CO_2 concentrations of ~ 30 % *v/v* and 10 – 20 % *v/v*, respectively, can be used as low–cost feedstock for *N. muscorum* cultivation.

A complete CO_2 consumption was recorded after *6.9* days of cultivation under a 30 % *v/v* CO_2 atmosphere (corresponding to an initial concentration of 525.0 ± 27.9 g CO_2 m^{-3}) (Fig. 6.3a), achieving a maximum biomass concentration of 733.3 ± 207.4 mg TSS L^{-1} at a pH of 10.5 ± 0.2. As expected, the production of biomass decreased when decreasing the CO_2 available in the headspace (Fig. 6.3b). Similarly, Bhati and Mallick (2016) reported an increase in the *N. muscorum* biomass yield from 620.0 ± 2.3 mg L^{-1} up to 880.6 ± 3.0 mg L^{-1} when increasing the content of the CO_2-enriched air supply from 2 to 15 % *v/v*. Considering a ratio of ~1.8 kg of CO_2 required per kg of biomass produced (assuming a biomass content of 50 % of carbon) (Posadas et al., 2017a), the potential of CO_2 fixation was 2.6 ± 0.0, 61.6 ± 6.8, 119.9 ± 19.3, 191.9 ± 46.0 and 165.6 ± 55.0 g m^{-3} d^{-1} under CO_2 concentrations of 0.03, 5, 10, 20 and 30 % *v/v*, respectively. Similar values to those observed in our study (~144 g m^{-3} d^{-1}) were reported by Li et al., (2020) when cultivating *Chlorella sp.* LAMB 31 under 40 % of CO_2, whereas a higher CO_2 fixation capacity was found by Bhati and Mallick (2016) in a photobioreactor inoculated with *N. muscorum*, with maximum values of 263 g m^{-3} d^{-1} at 10 % *v/v* CO_2-enriched air. It is important to note that the CO_2 fixation values here reported are based on the average biomass production of each batch test and not on the maximum biomass yield (which would correspond to the exponential growth phase), since biomass samples were only taken at the beginning and end of each test. Thus, higher CO_2 fixation rates are expected under continuous operation.

Test Series 1 demonstrated the tolerance of *N. muscorum* to high CO_2 concentrations in the gas phase, similar to those typically found in biogas (~30 % *v/v*), under N-sufficient conditions (Fig. 6.3a).
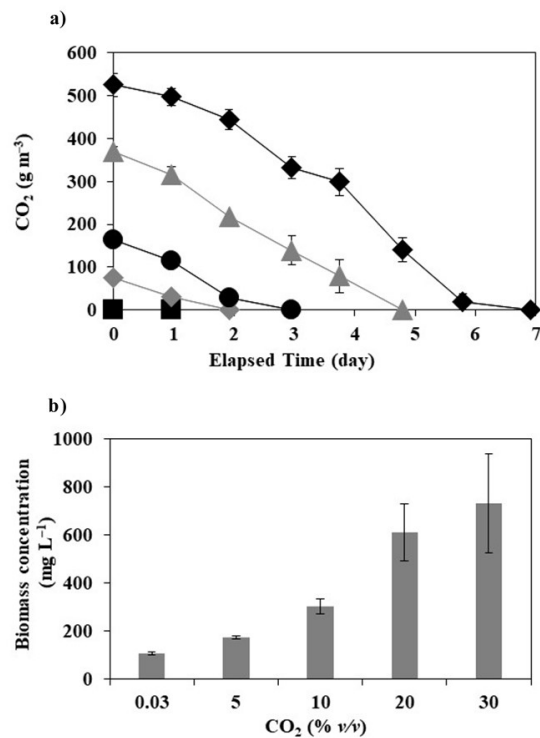
a)

b)

Fig. 6.3. a) Time of course of CO_2 concentration at different initial headspace compositions: 0.03 (square), 5 (grey diamond), 10 (circle), 20 (triangle) and 30 (black diamond) % v/v CO_2. b) Biomass concentration achieved by the end of Test Series 1 under different initial headspace compositions. Vertical bars represent standard deviations (± SD, n= 2 to 4).

However, the effect of H_2S on the growth of *N. muscorum* must also be assessed to ensure the possibility of using H_2S-loaded raw biogas as a C source. In this sense, the results obtained in Test Series 2 demonstrated that the presence of H_2S had a negative effect on the culture, inhibiting *N. muscorum* growth and therefore CO_2 abatement (Fig. 6.4a). This inhibitory effect was associated to the reduction in the pH due to the presence

of H_2S, which decreased from 5.9 ± 0.1 at the beginning of the experiment down to 4.9 ± 0.1 after 23.9 days of cultivation. Most cyanobacteria and microalgae achieve an optimal growth at pH 7 – 8, hence, a severe decrease in the pH modifies the enzymatic activity and energetics of the cells, resulting in a detrimental effect on their growth (Ángeles et al., 2019).

a)

b)
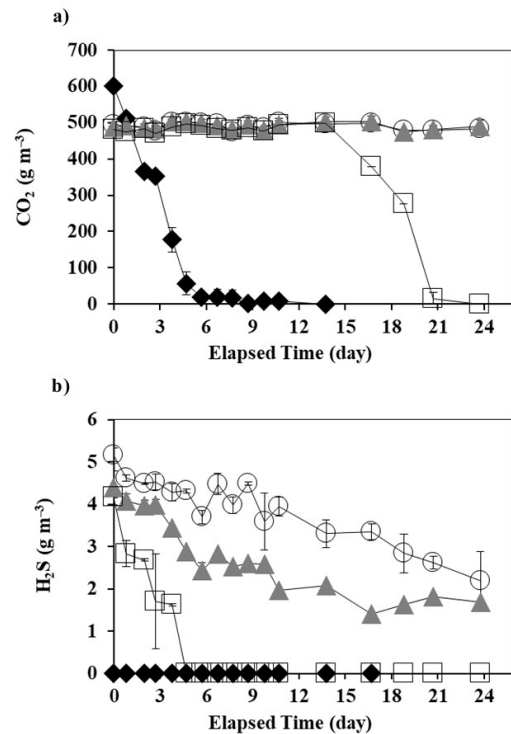
Fig. 6.4. Time course of a) CO_2 and b) H_2S concentration during Test Series 2 in biogas inoculated with *N. muscorum* (triangle), biogas inoculated with *N. muscorum* and activated sludge (square), a biotic control with H_2S-free biogas inoculated with N. muscorum (diamond) and an abiotic control (circle). Vertical bars represent standard deviations (± SD, n=2 to 4).

On the contrary, *N. muscorum* and bacteria symbiosis resulted in a complete consumption of CO_2 and H_2S (Figures 6.4a and 6.4b), achieving a final biomass

concentration of 467.5 ± 56.2 mg TSS L⁻¹ after 23.9 days of incubation.

6.3.2 Accumulation of value added compounds under N and P deprivation

As expected, the control bottle of Test Series 3 (i.e. N and P sufficient conditions), supported a rapid CO_2 abatement within less than 2 days, resulting in a final biomass concentration of 388.0 ± 22.6 mg TSS L⁻¹, which corresponded to a CO_2 fixation rate of 287.7 ± 4.0 g m⁻³ d⁻¹ (Fig. 6.5a). Similarly, under N-deprivation, the CO_2 concentration in the headspace was depleted to ~0.10 and 1.2 g CO_2 m⁻³ (CO_2 removals > 99 %) within 1.8 days of experiment at P concentrations of 2.5 and 1.1 mg $P-PO_4^{3-}$ L⁻¹ in the cultivation broth, respectively. A concomitant increase in the pH to 9.8 ± 0.9 and 9.1 ± 0.0 was recorded, together with an increase in the biomass concentration to 293.3 ± 37.7 and 333.3 ± 0.0 mg TSS L⁻¹ (corresponding to CO_2 fixation rates of 195.6 ± 39.5 and 241.0 ± 0.0 g m⁻³ d⁻¹, respectively). On the other hand, the cultures under N-deprivation and 0.2 mg $P-PO_4^{3-}$ showed a slightly lower CO_2 removal, with final values of 26.7 ± 21.2 g CO_2 m⁻³ in the headspace (removal efficiency of ~85 %) and a pH of 7.9 ± 0.0. Hence, a lower biomass concentration of 226.7 ± 37.7 mg TSS L⁻¹ and a CO_2 fixation of 122.2 ± 0.0 g m⁻³ d⁻¹ were recorded. The reduction in CO_2 consumption and biomass production under low P (below 0.2 mg $P-PO_4^{3-}$) concentration was

predictable since it is an essential nutrient for the metabolism of nucleic acids (RNA and DNA), membrane phospholipids and ATP in cyanobacteria (Markou et al., 2014).
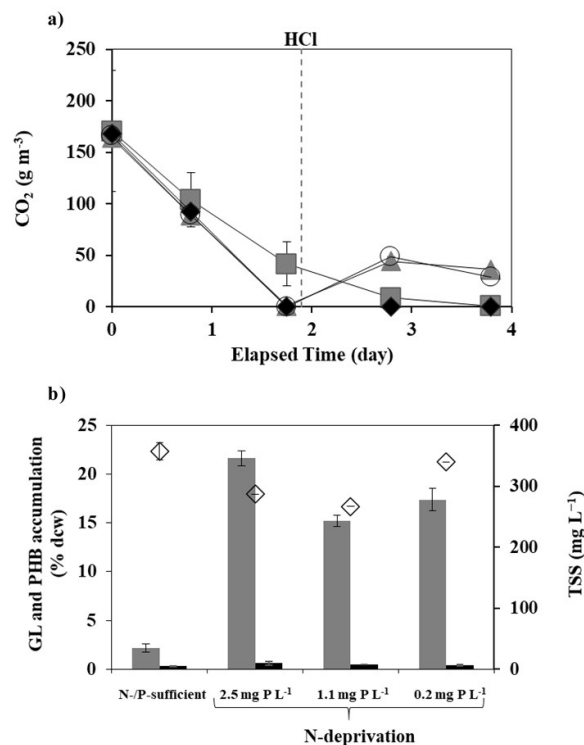

Fig. 6.5. a) Time course of CO_2 concentration under N- and P-sufficient condition (diamond), and under N-deprivation (10 mg N L⁻¹) and different P concentrations: 2.5 (circles), 1.1 (triangles) and 0.2 (squares) mg $P-PO_4^{3-}$ L⁻¹. b) Glycogen (GL) (grey bars) and PHB (dark bars) accumulation, and biomass concentration (white diamonds) recorded by day 3.8 under different N and P deprivation conditions in Test Series 3. Vertical bars represent standard deviations (± SD, n=2 to 4).

After the addition of HCl by day 1.8, the inorganic carbon remaining in the cultivation broth was desorbed into the headspace. Under N-sufficient conditions, the CO_2 was completely depleted,

although biomass concentration remained roughly constant at ~350 mg TSS L^{-1} by the end of the experimental period (Fig. 6.5a). A steady CO_2 decrease was observed under N deprivation and 0.2 mg P-PO_4^{3-} L^{-1}, with a concomitant increase in the TSS value from 226.7 ± 37.7 to 340.0 ± 0.0 mg TSS L^{-1}. On the other hand, the tests supplemented with 2.5 and 1.1 mg P-PO_4^{3-} L^{-1} showed neither a significant CO_2 consumption nor biomass growth.

Despite several studies have evidenced the capacity of *N. muscorum* to accumulate intracellular PHB up to 55 % dcw under nutrient limitation (Haase et al., 2012; Mallick et al., 2007b; Nishioka et al., 2001), insignificant PHB accumulation was recorded in this particular study (Fig. 6.5b), with maximum values of 0.6 ± 0.2 % dcw under P and N deprivation. These low values were attributed to the high inorganic carbon concentration available in the headspace, and therefore the high C:N ratio in the cultivation broth. On the contrary, *N. muscorum* was able to accumulate significant amounts of GL under N-deprivation, achieving maximum values of 21. 6 ± 0.8 % dcw at 2.5 mg P-PO_4^{3-} L^{-1}, while decreasing to 15.2 ± 0.6 and 17.3 ± 1.3 % dcw at lower P concentrations of 1.1 and 0.2 mg P-PO_4^{3-} L^{-1}, respectively. Despite the fact that P limitation might theoretically increase GL accumulation, these results suggested that P limitation below a certain concentration (N:P ratio > 4) even resulted in a detrimental effect on GL production.

Similar results were observed by De Philippis et al. (1992) for *Spirulina maxima* when cultivated under a pure CO_2 atmosphere and a pH level between 9.3 and 9.6. These authors also observed a negligible PHB accumulation of ~1.2 % dcw under either N or P deprivation, while GL concentrations up to 70 % dcw under N-deprivation and 23 % dcw under P-deprivation were achieved. Aikawa et al., (2014) also recorded the maximum GL productivity (up to 65 % dcw) in *Synechococcus* sp. PCC 7002 under a high light intensity of 600 µmol m^{-2} s^{-1}, high inorganic carbon concentration (2400 mg L^{-1}) and N-depleted conditions, whereas N supplementation resulted in a decrease in GL accumulation down to 2.2 ± 0.4 % dcw. In contrast, *Synechocystis* sp. PCC 6803 showed 36.8 and 13.5 % dcw of GL and PHB accumulation, respectively, when cultivated under N-deprivation and atmospheric CO_2 concentrations (0.03 % v/v) (Monshupanee and Incharoensakdi, 2014). Existing literature evidenced that the GL biosynthetic pathway competes with the PHB pathway at the level of the 3–phosphoglycerate (3–PG) pool for the accumulation of GL (Singh et al., 2017). In this sense, a high CO_2 concentration of 30 % v/v boosts the accumulation of 3–PG (Fig. 6.1), which enhances the activity of adenosine diphosphate (ADP)–glucose pyrophosphorylase (AGP), responsible of regulating cyanobacterial GL synthesis. Thus, a large part of the 3–PG pool is directed to GL synthesis compared to the PHB biosynthetic pathway, resulting in a lowered PHB production of 13.5 % dcw *vs*

GL production of 36.8 % dcw (Monshupanee and Incharoensakdi, 2014; Singh et al., 2017).

6.3.3 Influence of VFAs on the accumulation of value added compounds under nutrient deprivation

Prior addition of VFAs in Test Series 4, a rapid decrease in the CO_2 concentration was recorded under both N–sufficient and N-deprivation, achieving removals of 100 and ~95 % by day 2.4 of experiment, respectively, and biomass concentrations of 384.7 ± 13.4 and 326.4 ± 19.7 mg TSS L^{-1} (which corresponded to CO_2 fixation rates of 163.0 ± 21.1 and 129.5 ± 13.0 g m^{-3} d^{-1}, respectively) (Fig. 6.6a). A final pH of ~9.8 was obtained regardless of the N concentration. Following VFAs supplementation by day 3.3, no further CO_2 consumption was observed, likely due to the inhibition of *N. muscorum* driven by the low pH values after the addition of acetic (8.4 ± 0.6) and valeric (8.5 ± 1.1) acids. On the contrary, complete CO_2 consumption was recorded in both control bottles (with no VFAs supplementation). Under N-deprivation, acetic and valeric acid removals of 61.0 ± 36.3 % and 4.5 ± 0.7 %, respectively, were achieved by day 5.1, whereas negligible VFAs degradation was recorded during N-sufficient conditions.

As previously observed in Test Series 3, no significant PHB accumulation was observed under nutrient limitation at any of the conditions tested (Fig. 6.6b). Nevertheless, nutrients deprivation promoted the accumulation of GL, achieving maximum value of 29.9 ± 4.6 % dcw with the addition of acetic acid, and a slightly lower value of 29.3 ± 14.8 % dcw in the absence of VFAs. Surprisingly, the lowest GL accumulation (20.4 ± 11.8 % dcw) was recorded with the addition of valeric acid, which was in agreement with the limited degradation obtained for this VFA.

The results obtained in Test Series 4 exhibited that the addition of VFAs after CO_2 depletion in the headspace did not trigger a significant increase in the GL accumulation potential of *N. muscorum.* This could be attributed to the decrease in pH induced by the addition of VFA, which might hinder cyanobacterial activity and CO_2 fixation. Therefore, in Test Series 5, VFAs were supplemented at the beginning of the test and the pH was adjusted in order to avoid any inhibitory effect. As previously observed, nutrient sufficient controls completely consumed CO_2 within the first 2 days of the experiment (Fig. 6.6c), with a concomitant increase in the pH of the cultivation broth up to 10.8 ± 0.1. On the contrary, N and P deprivation resulted in a detrimental effect on CO_2 fixation, achieving a removal efficiency of 57.1 ± 2.9 % by day 5 of experiment. The presence of acetic acid further reduced CO_2 removal to 43.1 ± 8.7 %, however, the addition of valeric acid did not induce

such a severe inhibition on CO_2 consumption, reaching steady removals of 86.4 ± 17.6 % by day 5. Surprisingly, higher acetic acid removals were obtained compared to those of valeric acid, with values of 78.0 ± 21.0 % and 32.2 ± 7.3 % by day 5, respectively.
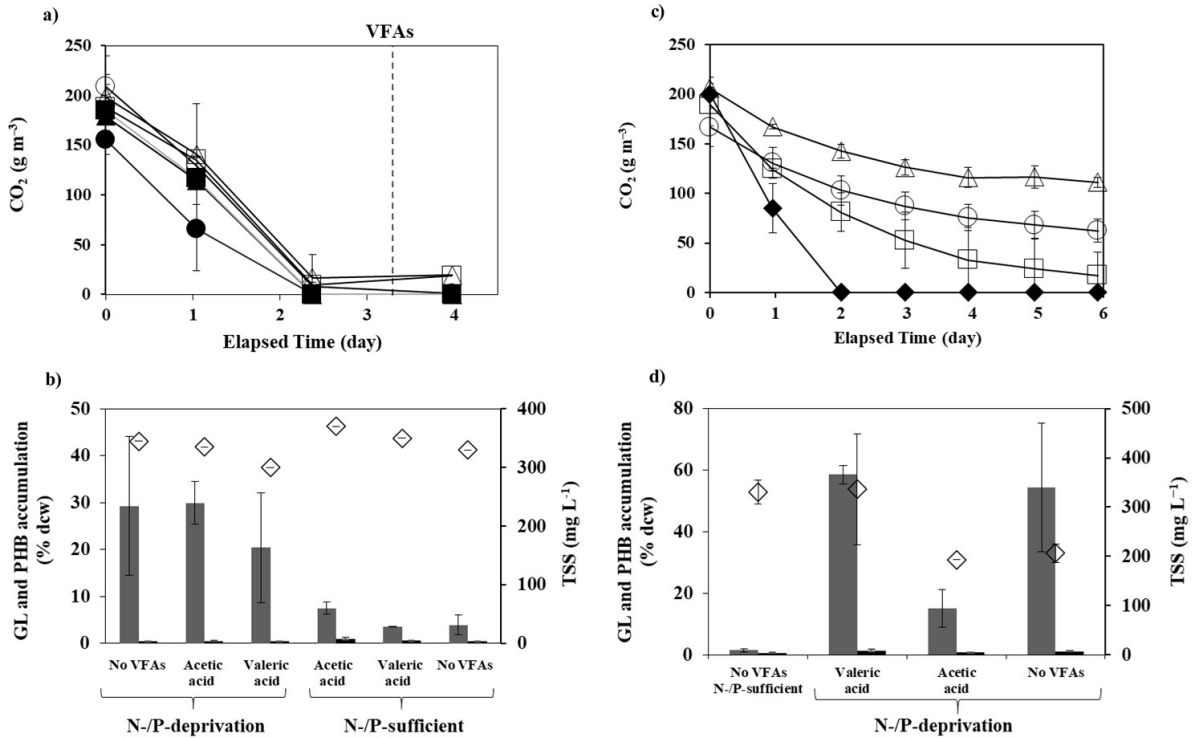

Fig. 6. a) Time course of CO_2 concentration in Test Series 4 under N-sufficient (empty symbols) and N-deprivation (filled symbols) conditions with no VFAs supplementation (circle) and supplemented with acetic (triangle) and valeric (square) acids. b) Glycogen (GL) (grey bars) and PHB (dark bars) accumulation and maximum biomass yield (white diamond) recorded by day 3.8 in Test series 4. c) Time course of CO_2 concentration in Test Series 5 under N- and P-sufficient condition (diamond), and under N- and P -deprivation with no VFAs supplementation (circle) and supplemented with acetic (triangle) and valeric (square) acids. d) Glycogen (grey bars) and PHB (dark bars) accumulation and maximum biomass yield (white diamond) recorded by day 5.0 in Test Series 5. Vertical bars represent standard deviations (± SD, n=2 to 4).

Nutrients deprivation resulted in an increase in GL accumulation from 1.5 ± 0.5 % up to 54.3 ± 20.8 % dcw (Fig. 6.6d). This higher GL production observed in Test Series 5 compared to Test Series 3 and 4 suggested that simultaneous N and P deprivation might be key to promote GL synthesis in *N. muscorum*. The addition of valeric acid not only boosted CO_2 fixation rates but also GL concentrations, reaching a maximum value of 58.5 ± 3.0 % dcw (Fig. 6.6d). On the contrary, the acetic acid-supplemented cultures under N-deprivation exhibited a considerably

lower GL content of 15.1 ± 6.2 % dcw. Finally, a negligible PHB accumulation (< 1 % dcw) was recorded regardless of the conditions tested. These results supported the potential of *N. muscorum* to fix CO_2 and accumulate significant concentrations of GL (compared to those of PHB) during photosynthetic biogas upgrading under nutrient deprivation and valeric acid supplementation. Similarly, Rueda et al. (2020) reported a maximum GL accumulation of 68.9 % dcw in *Synechocystis* sp. under nutrient deprivation and high dissolved IC (~122 mg L^{-1}) concentrations in the cultivation broth, whereas PHB accumulation was significantly lower (5% dcw). Likewise, the cyanobacteria strain *Synechocystis* sp. PCC 6714 reached a maximum GL accumulation of 49.8 % dcw using CO_2 as a carbon source under N and P deprivation, while PHB accumulation was ~12 % dcw (Kamravamanesh et al., 2019).

6.3.4 Biotechnological perspectives and practical applications

The results obtained in this study demonstrated the ability of *N. muscorum* to grow and accumulate GL using CO_2 from raw biogas as the carbon source. The subsequent biomass recovery process and the extraction and purification of the accumulated GL are key issues to ensure the scale-up potential of this technology. In this context, technologies such as filtration and centrifugation achieve concentrations up to 25 % of solids, while flocculation and flotation reach biomass concentrations ~4 times lower than centrifugation, although at reduced capital and operating costs (Menegazzo and Fonseca, 2019). On the other hand, alkaline hydrolysis is the most common method used for GL extraction in cyanobacteria, followed by acid hydrolysis, mechanical disruption of the cells, and thermolysis. To ensure a high degree of recovery and purification, GL precipitation with cold ethanol is also required after alkaline hydrolysis (Vidal and Venegas-Calerón, 2019). Once extracted, GL can be used as a low-cost carbohydrate source for bioethanol or for bio-based chemical production (Alam and Wang, 2019; Klein et al., 2015).

For instance, bioethanol production was investigated for marketing at industrial scale using *Chlorella vulgaris*, which reached a carbohydrate content of 55 % dcw resulting in a bioethanol yield of ~92 %. The techno-economic analysis of this technology showed a total bioethanol production cost of US$2.22 million per annum, a total bioethanol selling price of US$2.87 million per annum, and a total by-product selling price of US$1.6 million per annum (including raw materials for biofuels production except bioethanol). Moreover, the production of 220 tons of dry biomass of *Chlorella vulgaris* resulted in the absorption of 366.6 tons of CO_2, which represents a significant reduction of this GHG (Hossain et al., 2019).

Moreover, recent studies on genetic engineering have enhanced the PHB accumulation potential of certain cyanobacteria strains. For instance, a content of up to 35 % dcw of PHB was achieved in a genetically modified *Synechocystis* sp. PCC 6803 via transformation of the genes involved in the PHB synthesis pathway (Khetkorn et al., 2016). Despite the application of genetic and metabolic engineering may enhance the conversion of CO_2 into value added products in cyanobacteria (i.e. ethanol, hydrogen, glycerol, isobutanol, lactic acid, or PHB), it is still necessary to determine the biomass yields at an industrial scale and the financial viability of the process (Carpine et al., 2017).

Notwithstanding the numerous studies focused on exploring the conversion of CO_2 into marketable products by cyanobacteria, more research at laboratory and pilot scale is mandatory to determine the optimal conditions for the production of biomass and value added products. Further investigations at laboratory scale must address the potential of continuous CO_2-fixation from biogas coupled with GL production and extraction prior testing this technology at pilot scale. Moreover, the productivity of GL on the long term, which depends on both the GL accumulation percentage and the *N. muscorum* biomass productivity, must be also assessed to ensure the techno-economic feasibility of the process.

6.4 Conclusions

The capacity of *N. muscorum* to grow and accumulate value added compounds under high CO_2 concentrations in the gas phase, similar to those typically found in raw biogas from anaerobic digestion (~30 % v/v), was here demonstrated. Nitrogen-deprivation in the cultivation broth had no significant negative effect on CO_2 removals efficiencies (> 93 %), whereas nutrients limitation was necessary to promote GL synthesis. In this context, the maximum GL and PHB accumulation was achieved under N-/P-deprivation when 10% of the C supplemented as CO_2 was added as valeric acid, reaching values of 58.5 ± 3.0 and 1.4 ± 0.4 % dcw, respectively, at a biomass concentration of 336.0 ± 113.1 mg TSS L^{-1}. Despite the potential of *N. muscorum* to degrade VFAs, pH buffering was found crucial to avoid growth inhibition. In summary, *N. muscorum* could be a promising low-cost carbohydrate source for bioethanol or bio-based chemicals production, while simultaneously removing CO_2 biogas or flue gas.

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Conclusions and future work

Photosynthetic biogas upgrading has been successfully confirmed as a sustainable and environmentally friendly alternative that guarantees a high performance in the elimination of the main biogas contaminants (i.e., CO_2 and H_2S), while generating a valuable algal biomass from the nutrients contained in the wastewater.

In order to further optimize the quality of the biomethane, results from **Chapter 3** showed the potential of two novel operational strategies for reducing the N_2 and O_2 concentration in the upgraded biogas. The first operational strategy was based on a hollow fiber PDMS gas-liquid membrane to remove dissolved oxygen from the cultivation broth. This membrane unit reduced the content of N_2 and O_2 down to ~0.8 and ~0.1 % v/v, respectively, in the upgraded biogas. The second operational strategy increased the removal efficiency of CO_2 and H_2S when increasing the operating pressure in the biogas scrubbing column. Unfortunately, minimum overpressures of 2 atm were necessary to limit N_2 and O_2 desorption.

Photosynthetic biogas upgrading was also implemented in an enclosed PBR configuration coupled with the production of algal biomass in **Chapter 4**. This research demonstrated the positive effect of a high alkalinity in the cultivation broth of ~1700 mg L^{-1}, which supported > 96 % v/v of methane in the upgraded biogas, thus complying with the international regulations for methane injection into natural gas grids or use as vehicle fuel. The limited stripping from the cultivation broth in the scrubbing column provided satisfactory O_2 and N_2 contents in the upgraded biogas, which was favored by the enclosed configuration of the photobioreactor. After demonstrating the key role of the high alkalinity of the cultivation broth on biogas upgrading, process operation under nitrogen feast-famine cycles was implemented, which did not result in a detrimental effect on biomethane quality. Nitrogen deprivation in the cultivation broth for 24 and 48 h induced an increase in the carbohydrate content of the harvested biomass up to ~29 % dcw. In this context, a low microalgae diversity was recorded under process operation at high alkalinities and nitrogen limitation.

In **Chapter 5** the feasibility of continuous biogas upgrading in an enclosed tubular PBR was confirmed. Microalgae inhibition induced by H_2S build-up in the cultivation broth was prevented through the effective oxidation of H_2S during the dark period using the NO_3^- produced from NH_4^+ oxidation during the light period. The high alkalinity of the cultivation broth and the low operational L/G ratios in the biogas SC supported a complete elimination of H_2S and a limited desorption of N_2 and O_2.

However, lower CO_2 removals were recorded as a result of the acidification of the cultivation broth (pH ≤ 9) when raw biogas was supplied continuously 24 h per day. Biomass productivity in this study remained constant under the two biogas supply regimes tested. Interestingly, the intra-cellular carbohydrate content of microalgae increased by ~24 % under continuous biogas supply.

Photosynthetic upgrading of biogas by cyanobacteria coupled with the production of value added biomass constitutes a sustainable and environmentally friendly alternative to conventional physical-chemical methods that could boost the cost-effectiveness of CO_2 capture, while significantly reducing the production costs of these value added products. The potential of the filamentous N_2-fixing cyanobacterium *Nostoc muscorum* for CO_2 capture from high-strength gas streams (i.e., flue gas or biogas), combined with the accumulation of glycogen and polyhydroxybutyrate, was evaluated in **Chapter 6**. Glycogen contents of up to 60 % dcw were obtained under a 30 % *v/v* CO_2 atmosphere and nutrients deprivation. These results confirmed the potential of *N. muscorum* for using raw biogas as a low-cost carbon feedstock for carbohydrates production.

Despite the advances carried out in the present thesis towards the implementation of photosynthetic biogas upgrading in algal-bacterial photobioreactors combined with algal biomass valorization, further research is still necessary, as specified below:

- Despite the results confirmed the potential of the hollow fiber PDMS membrane to reduce the O_2 and N_2 content in the upgraded biogas, long-term testing of this strategy at pilot scale is necessary. Indeed, a long-term operation of the degassing membrane unit coupled to the HRAP- SC system is necessary to determine the membrane maintenance requirements and to estimate both the membrane lifetime and the energy demand at a representative scale.

- Evaluation of process performance under different liquid to biogas flowrate ratios when increasing the operating pressure in the biogas SC. A higher pressure entails both an increase in gas solubility (minimizing O_2 and N_2 desorption and boosting CO_2 uptake) and a reduction in the bubble size, and therefore, an increase in the specific area and the mass transfer coefficient (k_La) from the liquid to the gas phase.

- The evaluation of the energy consumption, emissions, and environmental impacts using a life cycle assessment approach of photosynthetic biogas

upgrading in an enclosed tubular PBR. This analysis will be crucial to assess the technical and environmental performance of photosynthetic biogas upgrading and to optimize the energy and CO_2 footprint.

- The investigation of factors promoting the accumulation of value added products without compromising biomass productivity during photosynthetic biogas upgrading, with the purpose of increase the economic sustainability of the process.

- The optimization of the operational parameters that guarantee the dominance of the desired microalgae or cyanobacteria species targeting at producing a specific marketable by-product without compromising the purity of the biomethane.

- Evaluation of the cost-competitiveness of biomass recovery to ensure the sustainability of the mass production of value added products coupled with biogas upgrading.

Chapter 8

About the author

Roxana Ángeles was born in Poza Rica de Hidalgo, Veracruz, Mexico. She studied Environmental Engineering and holds a MSc in Environmental Science from Veracruzana University, Mexico. Between 2008 and 2009, Roxana collaborated for 14 months with the research center of Biotechnology in Resource Management at the Institute of Ecology, INECOL, (Mexico), under the supervision of Professor Dr. Eugenia Olguin and Dr. Gloria Sanchez-Galvan. For the next three years, she worked as a supervisor in a facility of the International Company Stericycle, Inc. treating hazardous waste and wastewater from oil-drill processes. In February 2014, Roxana conducted a 6-month research stay at the Gas Treatment-Microalgae Research Group headed by Dr. Raúl Muñoz under the scientific supervision of Dr. Raquel Lebrero in the Environmental Technology Research Group (Department of Chemical Engineering and Environmental Technology, University of Valladolid, Spain). During the academic year 2015-2016, Roxana was the coordinator of the Degree in Environmental Engineering at the Superior Technological Institute "Martinez de la Torre" (Mexico).

During her early research career, Roxana was involved in several projects related to microalgae cultivation, studying the role of rhizospheric cyanobacteria in the removal of metals for bioremediation of metal-polluted mangroves and assessing the performance of microalgal-bacterial bioreactors for the treatment of volatile organic compounds (VOCs). In 2016, Roxana Angeles was awarded with a 48-month scholarship by the Mexican National Council for Science and Technology (CONACYT) and the Secretary of Energy (SENER) (Grant Agreement number 438909) to complete her PhD studies at the University of Valladolid within the Chemical and Environmental Engineering PhD Programme. Her research focused on the evaluation and optimization of microalgal-bacterial biogas upgrading in enclosed photobioreactors. Besides, her thesis explored new strategies to produce value added products by cyanobacteria and microalgae in order to improve the cost-effectiveness and environmental sustainability of the biogas upgrading process.

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Book Chapters

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Participation in international conferences

Oral presentations

- **Ángeles R,** Vega-Quiel, M., Batista, A., Muñoz, R., Lebrero, R. *Influence of biogas supply regime on photosynthetic biogas upgrading performance in an enclosed photobioreactor.* 9th Simposio de Becarios CONACYT en Europa. December 8th-10th, 2020. Strasbourg, France.

- **Ángeles R,** Gutiérrez J, Arnáiz E, Muñoz R, Lebrero R. *Evaluation and optimization of photosynthetic biogas upgrading in a tubular photobioreactor.* IWA Conference on Algal Technologies and Stabilitization Ponds for Wastewater Treatment and Resource Recovery-IWAlgae2019. July1-2st July, 2019. Valladolid, Spain.

- Rodero R, **Ángeles R,** García D, Lebrero R, García P, Muñoz R. *Procesos de microalgas como plataforma para la mejora de la eficiencia energética en EDARs.* Workshop NOVEDAR. April 3rd, 2017, Madrid, Spain.

- **Ángeles R,** Gutiérrez J, Arnáiz E, Muñoz R, Lebrero R. *Biogas upgrading and PHB accumulation in cyanobacteria.* 8th Simposio de Becarios CONACYT en Europa. April 3rd-5th, 2019. Strasbourg, France.

- **Ángeles R,** Gutiérrez J, Arnáiz E, Muñoz R, Lebrero R. *Biogas upgrading and PHAs accumulation in cyanobacterium.* EUALGAE – 3rd Training School: Microalgal bioeconomy in modern society. September 24-25th, 2018. Florence, Italy.

- **Ángeles R,** Oliva G, Zarra T, Naddeo V, Belgiorno V, Munoz R, Lebrero R. *Comparative evaluation of a biotrickling filter and a tubular photobioreactor for the continuous abatement of toluene.* NOSE 2018. September 9-12th, 2018. Milan, Italy.

- Oliva G., Zarra T., Naddeo V., Munoz R., Lebrero R., **Ángeles R.,** Belgiorno V. *Comparative analysis of AOPS and biological processes for the control of VOCs industrial emissions.* NOSE 2018. September 9-12th, 2018. Milan, Italy.

- Muñoz R, **Ángeles R,** Lebrero R. *Comparative evaluation of bacterial and algal airlifts for toluene degradation.* IWA Water and Industry. June 7-10th, 2015. Vasteras, Sweden.

Posters

- **Ángeles R,** Dominguez C, Arnáiz E, Palacio L, Prádanos P, Hernandez A, Muñoz R, Lebrero R. *Membrane Technology for O_2 and N_2 Removal During Biogas Upgrading in Microalgae Photobioreactor.* IWA Conference on Algal Technologies and Stabilitization Ponds for Wastewater Treatment and Resource Recovery-IWAlgae2019 July1st -2nd, 2019. Valladolid, Spain.

- **Ángeles R,** Gutiérrez J, Arnáiz E, Muñoz R, Lebrero R. *Biogas Upgrading coupled with PHB Accumulation in Nostoc muscorum: Effect of Volatile Fatty Acids (VFAs) Addition.* IWA the International Water Association, 16th IWA World Conference on Anaerobic Digestion. June 23-27th, 2019. Delft, The Netherlands.

- Olguín E J, **Ángeles R,** Sánchez-Galván G. *Biosorption of Lead by dry biomass of Leptolyngbya crossbyana: physico-chemical characterization of biomass and q_{max} determination.* 4th Congress of the International Society for Applied Phycology. June 19-24 th, 2011. Halifax, Canada.

- Olguín E J, **Ángeles R,** Sánchez-Galván G. *Capacidad de adsorción de Pb^{2+} por una cianobacteria rizosférica del mangle negro (Avicennia germinasns)* Congress of Latin American Society Environmental and Algal Biotechnologies. December 5-9th, 2010. Cancún, México.

Fellowships

- Research internship grant from Fundación Carolina (Spain). Metropolitan Autonomous University (Mexico). January 2 nd – June 29 th.

- Excellence Grant. Sistema Estatal de Becas del Gobierno del Estado de Veracruz de Ignacio de la Llave (Mexico). September 2019.

- Grant for participating in the international conference: *"8th Simposio Becarios CONACYT en Europa. April 3rd-5th, 2019. Strasbourg, France"* (UVa fellowship). April 2019.

- Training School. COST Association, International non-for-profit organization. European Cooperation in science & technology. Host institution: CNR-Area di Ricerca di Firenze, Instituto per lo Studio degli Ecosistemi. September 24 – 25th, 2018. Florence, Italy.

- Predoctoral researcher Fellowship. CONACYT-SENER, Mexico. 2016 – 2020.

- Research internships grant from CONACYT, Mexico. Valladolid University (Spain). February 1st – July 15th, 2014.
- Master Fellowship. CONACYT, Mexico. August 2014 – July 2016.

Participation in Research Projects

- Ayudas a la investigación Fundación Domingo Martínez. Área de Materiales 2018. *Nuevos procesos de microalgas y bacterias para la bioconversión de CO_2 de biogás a biopolímeros acoplada a la generación de biometano.* Fundación Domingo Martínez. 2018-2021. PI: R. Muñoz, University of Valladolid (Spain).
- Project CONACYT-104173 of the SIN-Estudiantes-2008-01. *Evaluación de la capacidad de bioadsorción de plomo de una cianobacterias aisladas de pneumatóforos de Avicennia germinans.* PI: E. Olguin, INECOL, A. C. (Mexico).

Teaching and students mentoring

- Co-supervisor of an international researcher from the Department of Chemistry, Autonomous University of Chiriqui (Panamá). Student: Miguel Vega (January-March 2020). Title: *Influence of biogas supply regime on photosynthetic biogas upgrading performance in an enclosed photobioreactor.*
- Co-supervisor of a Final Degree Project in Chemical Engineering Degree at University of Valladolid. Student: Ángel Rodríguez Herrero (April - July 2019). Project title: *Estudio y optimización de la operación de fotobiorreactores para el upgrading de biogás.* http://uvadoc.uva.es/handle/10324/37800
- Co-supervisor of a Final Degree Project in Chemical Engineering Degree at University of Valladolid. Student: Julia Gutiérrez Abadía (October 2019-January 2020). Project title: *Optimización del proceso de eliminación de CO_2 y acumulación simultánea de PHAs en Nostoc Muscorum.* http://uvadoc.uva.es/handle/10324/40510
- Co-supervisor of a Master Thesis in the Master of Environmental Engineering at University of Valladolid. Student: Christian Domínguez (September 2018-march 2019). Thesis title: *Tecnología de membranas para la eliminación de*

O$_2$ y N$_2$ durante el acondicionamiento de biogás en fotobiorreactores de microalgas. http://uvadoc.uva.es/handle/10324/37375

- Co-supervisor of an international researcher from Popular Autonomous University of the State of Puebla (México). Student: Ingrid Mejia (August-December 2018). Title: *Tecnología de membranas para la eliminación de O$_2$ y N$_2$ durante el acondicionamiento de biogás en fotobiorreactores de microalgas.*

Outreach activities

- Member of the Institute of the Sustainable Processes (ISP) of the University of Valladolid.
- Member of the Organizing Committee: *IWA Conference on Algal Technologies and Stabilitization Ponds for Wastewater Treatment and Resource Recovery-IWAlgae2019.* July1st-2nd, 2019. Valladolid, Spain.
- Member of the Organizing Committee: *IV Conferencia Internacional sobre Gestión de Olores y COVs en el Medio Ambiente.* September 20- 21st, 2017. Valladolid, Spain.

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