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PROGRAMA DE DOCTORADO EN
INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

Optimization of piggery wastewater treatment with purple phototrophic bacteria

Presentada por **Cristian Andrés Sepúlveda Muñoz** para optar al grado de
Doctor por la Universidad de Valladolid

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Resumen

La creciente población humana en el mundo conlleva futuros retos para el desarrollo sostenible de la humanidad. Esta expansión demográfica representará cambios globales generados por la actividad antropogénica en el planeta Tierra, involucrando impactos negativos en la biosfera, atmosfera, criósfera e hidrosfera. En particular, la hidrosfera será afectada por el incremento de la contaminación de aguas superficiales y subterráneas, y un aumento en la eutrofización de estas por un manejo inadecuado de las aguas residuales. Así, el desarrollo de nuevas tecnologías para la reducción de la contaminación en aguas residuales que posibilite un desarrollo sostenible de la humanidad representa un desafío científico-tecnológico para las próximas décadas.

En este sentido, el tratamiento eficiente de las aguas residuales en ciudades, industria y agricultura, necesita el desarrollo de soluciones innovadoras para reducir la contaminación generada por la intensa actividad antropogénica. Específicamente, la necesidad de satisfacer la creciente demanda de proteína animal ha generado un aumento en la ganadería intensiva, con el consiguiente aumento en la producción de aguas residuales con altas concentraciones de materia orgánica, nitrógeno y fosforo. En particular, las aguas residuales de explotaciones porcinas (PWW, por sus siglas en inglés) se caracterizan por su alta concentración de materia orgánica y nitrógeno en forma de amonio, y por sus molestos olores.

Tradicionalmente, las PWW han sido tratadas mediante su disposición en lagunas facultativas abiertas, desde las que se emiten gases de efecto invernadero como CO_2, CH_4, NH_3 y H_2S hacia la atmosfera debido a su configuración abierta. Otra tecnología utilizada para el tratamiento de estas aguas residuales es el proceso de digestión anaerobia en biorreactores cerrados, la cual es capaz de generar metano (CH_4) como subproducto, un gas de alto valor económico y energético. Sin embargo, este proceso es solo capaz de eliminar el carbono presente en PWW, generando aguas residuales con un alto contenido de nitrógeno y fosforo que no es asimilado en el proceso. Por último, los sistemas de lodos activos conllevan una eliminación efectiva de carbono, nitrógeno y fósforo con altos costes energéticos y una destrucción de los nutrientes presentes en las PWW.

Resumen

En la actualidad se ha propuesto el uso de microorganismos fotosintéticos para el tratamiento de PWW con bajos costes de operación y una recuperación del carbono, nitrógeno y fósforo presente en estas aguas residuales. Estos microorganismos son capaces de absorber la radiación solar a través del proceso de fotosíntesis para la obtención de energía, utilizada para su crecimiento y asimilación de nutrientes en diferentes aguas residuales. Las bacterias fototróficas púrpuras (PPB, por sus siglas en inglés) constituyen los microorganismos fotosintéticos con el metabolismo más versátil en la naturaleza. Las PPB son capaces de crecer de forma fototrófica y quimiotrófica, absorbiendo energía de la radiación solar o de la degradación de compuestos orgánicos, respectivamente. Además, son microorganismos heterotróficos capaces de degradar numerosos compuestos orgánicos y de crecer mediante un metabolismo autótrofo, fijando dióxido de carbono (CO_2) y nitrógeno (N_2) desde la atmosfera. Las PPB pueden crecer tanto en condiciones anaerobias como en condiciones aerobias, mediante fotoheterotrofía o quimioautotrofía, respectivamente. Por otra parte, las microalgas representan los microorganismos fotosintéticos más estudiados en los últimos años, debido a su alta tasa de crecimiento, a su capacidad para fijar CO_2 y al alto potencial de valorización de su biomasa. Tanto el grupo de las PPB como microalgas presentan especies con capacidades metabólicas extraordinarias, capaces de crecer a bajas o extremadamente altas temperaturas, en diferentes rangos de pH (ácidos y alcalinos) y salinidad, y en presencia o ausencia de oxígeno, confiriéndoles un alto potencial para el tratamiento de diferentes aguas residuales.

En este contexto, en el capítulo 1 se evaluó la influencia de las cargas de aguas residuales de explotaciones porcinas, la dosificación de aire, suplementación de $CO_2/NaHCO_3^-$ y el control de pH en el tratamiento de PWW utilizando cultivos mixtos de PPB en fotobiorreactores en lote. Las PPB fueron capaces de crecer bajo radiación infrarroja en diluciones de PWW de 1:5, 1:10 y 1:15, inhibiéndose el crecimiento de las PPB con purines de cerdo no diluidos. En condiciones de alta dilución de PWW se favoreció el crecimiento de las PPB y las eficiencias de remoción de carbono y nitrógeno, debido a una mayor penetración de la radiación en el caldo de cultivo bacteriano, que favoreció la fotosíntesis junto a una disminución en los efectos tóxicos por las altas concentraciones de amonio presente en las PWW. Las PPB realizaron un eficiente tratamiento de PWW en condiciones anaerobias y aerobias. Sin embargo, bajo condiciones aerobias se registró una mayor pérdida de nutrientes por volatilización en

comparación al crecimiento en condiciones anaerobias, donde el principal mecanismo de remoción fue la asimilación de nitrógeno en forma de biomasa de PPB. La suplementación de CO_2 resultó en una eficiente remoción de nutrientes por parte de las PPB, mientras que el metabolismo de las PPB no se vio afectado por falta de elementos traza durante el tratamiento de PWW. Finalmente, el control de pH fue un parámetro clave para una eficiente remoción de nutrientes mediante PPB durante el tratamiento de PWW, mejorando su crecimiento y remociones de carbono y nitrógeno por el control de pH realizado por adición tanto de HCl como de CO_2. Las PPB fueron capaces de llevar a cabo una eficiente asimilación de nutrientes de PWW, siendo la suplementación de CO_2 y sus efectos beneficiosos en el control de pH claves para el crecimiento de PPB y para un tratamiento eficiente de PWW.

En el capítulo 2, se evaluó la influencia de diferentes tipos de radiación (radiación fotosintéticamente activa (PAR), radiación infrarroja cercana (NIR) y PAR filtrada utilizando un filtro UV-VIS), la temperatura (13 °C y 30 °C), el tipo de metabolismo (fotoheterotrófico y quimioheterotrófico), el tipo de inoculo (cultivos mixtos y cepa pura de *Rhodopseudomonas palustris*) y la carga de agua residual (diluciones de 1:5 y 1:10) en la eficiencia del tratamiento de PWW utilizando PPB en fotobiorreactores operados en modo discontinuo. Las PPB fueron capaces de crecer bajo las diferentes fuentes de radiación evaluadas, realizando un tratamiento eficiente de PWW. El uso de PAR filtrada por filtro UV-VIS indujo tanto un alto contenido de bacterioclorofila en PPB como la mayor eficiencia de remoción de carbono orgánico total (TOC-REs = 74%). Los cultivos mixtos de PPB presentaron TOC-REs y eficiencias de remoción de nitrógeno total (TN-REs) a baja temperatura (71% y 45%, respectivamente) similares en comparación con las registradas a una temperatura de 30 °C (73% y 37%, respectivamente), sin exhibir una disminución en la producción de biomasa de PPB. Las PPB no fueron capaces de crecer en oscuridad quimioheterotróficamente durante el tratamiento de PWW. Por otra parte, los cultivos mixtos de PPB consiguieron una mayor tasa de asimilación de nutrientes que *R. palustris*, exhibiendo una asimilación total de los ácidos grasos volátiles presentes en PWW diluido 10 veces. Las PPB fueron capaces de crecer y realizar una remoción de nutrientes eficiente, independientemente del tipo de radiación y la temperatura, exhibiendo una alta eficiencia de remoción utilizando cultivos mixtos de PPB en el tratamiento de PWW.

En el capítulo 3, se evalúo el rendimiento a largo plazo (450 días) de fotobiorreactores abiertos y cerrados operados en modo continuo durante el tratamiento de PWW a un tiempo de retención hidráulico de 7 días. La influencia de diluciones de PWW de 1:4 y 1:8, la relación de área iluminada/volumen del fotobiorreactor, la implementación de sedimentación y recirculación de la biomasa, y las intensidades de radiación infrarroja en la remoción de carbono y nitrógeno en PWW utilizando PPB fueron evaluadas. El incremento en la dilución de PWW de 1:4 a 1:8 no conllevó mejoras en la eficiencia de remoción de carbono orgánico total (TOC-RE) en el fotobiorreactor abierto (87% versus 89%), pero si un incremento significativo en las TOC-RE en el fotobiorreactor cerrado (de un 73% hasta un 80%). El aumento en el área iluminada/volumen de los fotobiorreactores incrementó las eficiencias de remoción de nitrógeno total (TN-RE) hasta un 99% y un 49% en el fotobiorreactor abierto y cerrado, respectivamente, con un aumento inherente en la temperatura en ambos sistemas. Sin embargo, el control de temperatura no mejoró significativamente el tratamiento de PWW. La sedimentación y recirculación de la biomasa resultó en mejoras en la TOC-RE finales de un 80% y TN-RE de un 90% en el fotobiorreactor cerrado. El incremento en la radiación infrarroja de 100 a 300 W m^{-2} fomentó el crecimiento de PPB. Se registraron altas pérdidas de agua por evaporación en el fotobiorreactor abierto (deteriorando la calidad del efluente), donde la volatilización de CO_2 y NH_3 fueron identificados como el principal mecanismo de remoción de carbono y nitrógeno. Tanto el fotobiorreactor abierto como el cerrado fueron eficientes en la eliminación de carbono en PWW, siendo mayor la eliminación de nitrógeno en el fotobiorreactor abierto y experimentado menores tasas de evaporación y un mayor crecimiento de PPB el fotobiorreactor cerrado.

Finalmente, en el capítulo 4 se evalúo el potencial de una innovadora configuración de reactores compuesta de un fotobiorreactor anaerobio de PPB (PPB-PBR) acoplado secuencialmente a un fotobiorreactor aerobio de microalgas-bacterias (MB-PBR) durante el tratamiento de PWW en modo continuo de operación. Se evaluaron los efectos del tiempo de retención hidráulico (HRT) y de las intensidades de radiación infrarroja cercana (NIR) en el PPB-PBR en la eliminación y recuperación de carbón y nitrógeno utilizando balances de materia y una completa caracterización de las comunidades bacterianas y de microalgas en los fotobiorreactores. Las eficiencias de remoción máximas de carbono orgánico total disuelto (TOC-RE) y nitrógeno total disuelto (TN-RE) (91% y 82%, respectivamente) fueron registradas a un HRT de 12.2

días. La reducción del HRT a 6.2 días conllevó una disminución en la TOC-RE y TN-RE en ambos fotobiorreactores. Sin embargo, el aumento de NIR en el PPB-PBR mejoró la TOC-RE, contribuyendo en una recuperación global de carbono del 67% a través de su asimilación en forma de biomasa de PPB. El PPB-PBR fue muy eficiente en la asimilación de carbono, mientras que el MB-PBR conllevó altas remociones de nitrógeno y de los sólidos suspendidos totales (63% y 76%, respectivamente). La bacteria *Rhodopseudomonas* sp. fue dominante en el caldo de cultivo del PPB-PBR, representando un 54% de la población bacteriana, apoyado por el alto HRT y el aumento de la NIR. Las condiciones ambientales y de operación en el MB-PBR secuencial favorecieron el dominio de *Mychonastes homosphaera*. Esta investigación demostró, por primera vez, la alta eficiencia de los sistemas secuenciales de PPB y microalgas para el tratamiento de PWW.

Los resultados obtenidos en esta tesis demuestran que un tratamiento eficiente de las aguas residuales de explotaciones porcinas es posible mediante el uso de microorganismos fotosintéticos (bacterias fototróficas púrpuras y microalgas).

Abstract

The increase in human population in the world entails future challenges for the sustainable development of mankind. This demographic expansion will represent global changes generated by anthropogenic activity on planet Earth, involving negative impacts on the biosphere, atmosphere, cryosphere and hydrosphere. In particular, the hydrosphere will be affected by the increased contamination of surface water and groundwater, and an increase in their eutrophication due to inadequate management of wastewaters. Thus, the generation of new biotechnologies to reduce pollution and improve the sustainable development of humanity will be a challenge for the next few decades.

In this sense, efficient wastewater treatment in cities, industry and agriculture requires the development of innovative solutions to reduce the pollution generated by the intense anthropogenic activity. Thus, the need for meeting the increasing demand for animal protein has led to an increase in intensive livestock farming, with the subsequent increase in the generation of wastewaters containing high organic matter, nitrogen and phosphorus concentrations. In particular, piggery wastewaters (PWW) are characterized by their high concentrations of organic matter and nitrogen in the form of ammonium, and by their odour nuisance.

Traditionally, PWW has been treated by disposal in open anaerobic lakes, with the subsequent production of high concentrations of greenhouse gases such as CO_2, CH_4, NH_3 and H_2S to the atmosphere due to their open configuration. Another technology used for the treatment of this type of wastewaters is anaerobic digestion in enclosed bioreactors, which is capable of generating methane (CH_4) as a byproduct, a gas with high economic and energy value. However, this process is only capable of removing the carbon present in PWW, generating an effluent with a high nitrogen and phosphorus content that is not assimilated in the process. Lastly, activated sludge systems entail an effective removal of carbon, nitrogen and phosphorous with a high energy demand and a destruction of the nutrients present in PWW.

Nowadays, the use of photosynthetic microorganisms for PWW treatment at low operating costs and with a recovery of carbon, nitrogen and phosphorous has been proposed. These microorganisms are capable of absorbing solar radiation through the photosynthesis process to obtain energy, which is used for their growth and nutrients assimilation from different wastewaters. Purple phototrophic bacteria (PPB) represent the photosynthetic microorganisms with the most versatile metabolism in nature. PPB can grow phototrophically and chemotrophically, absorbing energy from solar radiation or from the degradation of organic compounds, respectively. In addition, PPB are heterotrophic microorganisms capable of degrading different organic compounds and also able to grow using an autotrophic metabolism, fixing carbon dioxide (CO_2) and nitrogen (N_2) from the atmosphere. PPB can grow both under anaerobic and aerobic conditions, through photoheterotrophy or chemoautotrophy, respectively. On the other hand, microalgae represent the most studied photosynthetic microorganisms in recent years, due to their high growth rate, capacity to fix CO_2 and to the high valorization potential of their biomass. Both PPB and microalgae have species with extraordinary metabolic capacities, capable of growing at low or extremely high temperatures, under different pH ranges (acidic and alkaline), high salinity and in the presence or absence of oxygen, which supports their extraordinary metabolism for the treatment of multiple wastewaters.

In this context, Chapter 1 evaluated the influence of PWW load, air dosing, $CO_2/NaHCO_3^-$ supplementation and pH control on PWW treatment by mixed cultures of PPB in batch photobioreactors. PPB were able to grow under infrared irradiation in 1:5, 1:10 and 1:15 PWW dilutions, undiluted PWW inhibiting PPB growth. Under high PWW dilutions, PPB growth and carbon and nitrogen removal efficiencies were favoured, due to a higher penetration of the radiation into the cultivation broth, which enhanced photosynthesis and decreased the toxic effects caused by the high ammonium concentrations present in the PWW. PPB performed an efficient PWW treatment under anaerobic and aerobic conditions. However, under aerobic conditions an increased nutrients loss by stripping was recorded compared to the tests conducted under anaerobic conditions, where the main removal mechanism was nitrogen assimilation in the form of PPB biomass. CO_2 supplementation resulted in an efficient nutrient removal by PPB, while PPB metabolisms was not affected by lack of trace elements during PWW treatment. Finally, pH control was a key parameter for an efficient nutrient removal by PPB during PWW treatment, improving PPB growth, carbon and nitrogen removals by pH control via addition of both HCl and CO_2. PPB were able to perform and efficient nutrient assimilation from PWW, CO_2 supplementation and its beneficial effects on pH control being key to PPB growth and efficient PWW treatment.

In chapter 2, the influence of the type of radiation (photosynthetically active radiation (PAR), near-infrared radiation (NIR) and PAR filtered using a UV-VIS filter), temperature (13 °C and 30 °C), metabolism (photoheterotrophic and chemoheterotrophic), type of inoculum (mixed cultures and strain *Rhodopseudomonas palustris*) and wastewater load (1:5 and 1:10 dilutions) during PWW treatment by PPB in batch photobioreactors was evaluated. PPB were able to grow under the different radiation sources tested, performing an efficient PWW treatment. The use of UV-VIS filtered PAR supported both a high bacteriochlorophyll content in PPB and the highest total organic carbon removal (TOC-RE = 74%). Interestingly, PPB exhibited TOC-REs and total nitrogen removal efficiencies (TN-REs) at low temperature (71% and 45%, respectively) similar to those recorded at a temperature of 30 °C (73% and 37%, respectively), without exhibiting a decrease in PPB biomass production. PPB were not able to grow in darkness chemoheterotrophically during PWW treatment. On the other hand, mixed cultures of PPB achieved a higher nutrient assimilation rate than *R. palustris*, exhibiting a total assimilation of the volatile fatty acids present in 10-fold diluted PWW. PPB were able to grow and perform an efficient nutrient removal, regardless of the type of radiation and temperatures, exhibiting a high removal efficiency using mixed cultures of PPB during PWW treatment.

In chapter 3, the long-term performance (450 days) of open and closed photobioreactors operated in continuous mode during PWW treatment at a hydraulic retention time of 7 days was evaluated. The influence of 1:4 and 1:8 PWW dilutions, ratio of illuminated area/volume of the photobioreactor, biomass settling and recirculation, and infrared radiation intensities on the removal of carbon and nitrogen from PWW by PPB were evaluated. The increase in PWW dilution from 1:4 to 1:8 did not entail higher total organic carbon removal efficiency (TOC-RE) in the open photobioreactor (87% versus 89%), but a significant increase in the TOC-RE in the closed photobioreactor (from 73% to 80%). The increase in the illuminated area/volume of the photobioreactors increased total nitrogen removal efficiencies (TN-RE) up to 99% and 49% in the open and closed photobioreactor, respectively, with a concomitant increase in the temperature of both systems. However, temperature control did not mediate a significant enhancement in

PWW treatment. Biomass settling and recirculation resulted in higher final TOC-RE of 80% and TN-RE of 90% in the closed photobioreactor. The increase in infrared radiation from 100 to 300 W m^{-2} fostered PPB growth. High water evaporation losses were recorded in the open photobioreactor (deteriorating effluent quality), where CO_2 and NH_3 volatilization were identified as the main mechanism of carbon and nitrogen removal. Both open and closed photobioreactor were efficient in carbon removal from PWW, enhancing nitrogen removal in the open photobioreactor and exhibiting lower evaporation rates and higher PPB growth in the closed photobioreactor.

Finally, the potential of an innovative configuration composed of an anaerobic PPB photobioreactor (PPB-PBR) sequentially coupled to an aerobic microalgae-bacteria photobioreactor (MB-PBR) was assessed during the PWW treatment under continuous operation in Chapter 4. The effects of hydraulic retention time (HRT) and intensities of near-infrared radiation (NIR) in the PPB-PBR were evaluated using mass balances and a complete characterization of the bacterial and microalgal communities in the photobioreactors. Maximum removal efficiencies of total dissolved organic carbon (TOC-RE) and total dissolved nitrogen (TN-RE) of 91% and 82%, respectively, were recorded at an HRT of 12.2 days. The decrease in HRT to 6.2 days reduced TOC-RE and TN-RE in both photobioreactors. However, the increase in NIR in the PPB-PBR enhanced TOC-RE, contributing to a global carbon recovery of 67% via assimilation in the form of PPB biomass. PPB-PBR was highly efficient in carbon assimilation, while MB-PBR supported high nitrogen and total suspended solids removals (63% and 76%, respectively). The culture broth of PPB-PBR was dominated by the bacteria *Rhodopseudomonas* sp., which represented up to 54% of the total bacterial population, supported by the high HRT and increased NIR. The environmental and operational conditions set in the sequential MB-PBR favoured the dominance of *Mychonastes homosphaera*. This research demonstrated, for the first time, the high efficiency of the sequential PPB and microalgae systems for the treatment of PWW.

The results obtained in this thesis demonstrate that an efficient treatment of piggery wastewater using photosynthetic microorganisms (purple phototrophic bacteria and microalgae) is feasible.

List of publications

Paper 1: **Cristian A. Sepúlveda-Muñoz**, Ignacio de Godos, Daniel Puyol, Raúl Muñoz. (2020). A systematic optimization of piggery wastewater treatment with purple phototrophic bacteria. *Chemosphere*. 253: 126621.

Paper 2: **Cristian A. Sepúlveda-Muñoz**, Andrés F. Torres-Franco, Ignacio de Godos, Raúl Muñoz. (2022). Exploring the metabolic capabilities of purple phototrophic bacteria during piggery wastewater treatment. *submitted for publication in J Water Process Eng*.

Paper 3: **Cristian A. Sepúlveda-Muñoz**, Roxana Ángeles, Ignacio de Godos, Raúl Muñoz. (2020). Comparative evaluation of continuous piggery wastewater treatment in open and closed purple phototrophic bacteria-based photobioreactors. *J Water Process Eng*. 38: 101608.

Paper 4: **Cristian A. Sepúlveda-Muñoz**, Gorka Hontiyuelo, Saúl Blanco, Andrés F. Torres-Franco, Raúl Muñoz. (2022). Photosynthetic treatment of piggery wastewater in sequential purple phototrophic bacteria and microalgae-bacteria photobioreactors. *J Water Process Eng*. 47: 102825.

Contribution to the papers included in the thesis

Paper 1: In this research, I was responsible for the design, start-up and monitoring of the experimental batch tests under the supervision of Dr. Raúl Muñoz. I was responsible for the evaluation of the results and prepared the manuscript under the supervision of Dr. Raúl Muñoz, Dr. Ignacio de Godos and Dr. Daniel Puyol.

Paper 2: During this research, I was responsible for the design, start-up, operation of the experimental set-up and evaluation of the results under the supervision of Dr. Raúl Muñoz. I prepared the manuscript under the supervision of Dr. Raúl Muñoz, Dr. Ignacio de Godos and Dr. Andrés F. Torres-Franco.

Paper 3: In this work, I was responsible for the start-up, operation of the experimental set-up and results evaluation under the supervision of Dr. Raúl Muñoz. I was also in charge of the manuscript writing under the supervision of Dr. Raúl Muñoz and Dr. Ignacio de Godos. Roxana Ángeles assisted in photobioreactor monitoring.

Paper 4: During this research, I was responsible for the design, start-up and monitoring of the continuous photobioreactors under the supervision of Dr. Raúl Muñoz. I was responsible for the evaluation of the results and prepared the manuscript under the supervision of Dr. Raúl Muñoz and Dr. Andrés F. Torres-Franco. Dr. Saúl Blanco performed the analysis of microalgae identification and Gorka Hontiyuelo assisted in photobioreactors monitoring.

Introduction

Introduction

1.1. *Pollution and wastewater*

The human population in the world reached 7.7 billion in 2019 and recent projections suggested an average of population up to 9.7 billion by 2050, which represents future challenges for the sustainable development for mankind (Fig. 1). This demographic expansion will entail global changes generated by an intensive anthropogenic activity on the Earth, which involves negatives impacts in the atmosphere due to the intense emission of greenhouse gases, in the cryosphere due to the melting of ice fields, in the land surface cause by soil pollution and desertification, in the biosphere due to a loss of biodiversity and species extinction, and in the hydrosphere due to eutrophication and pollution of surface water and groundwater (Moss et al., 2010). The development of new technologies that reduce pollution and pave the way towards a sustainable development at a global scale represents a severe challenge nowadays. In this context, multiple technologies have been engineered in recent years to reduce the pollution generated by anthropogenic activity. These technologies include greenhouse gas capture (D'Alessandro et al., 2010; Olah et al., 2011), new renewable energy sources with special focus on solar energy and biofuels (Demirbas, 2007; Lewis and Nocera, 2006), sustainable farmland and livestock management (Hole et al., 2005) and an extensive innovation in wastewater treatment technology (Cai et al., 2013; Hülsen et al., 2022).

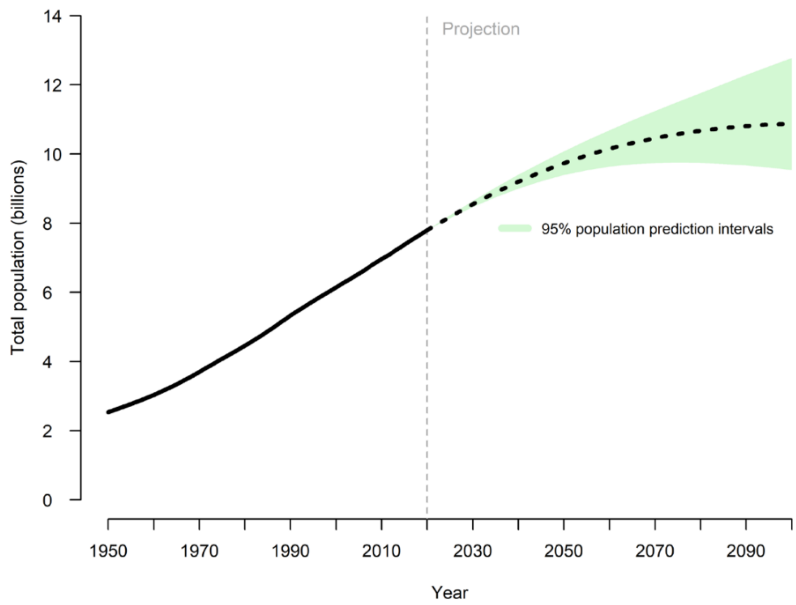

Figure 1. Evolution and estimation of World human population (adapted from World population prospects 2019, United Nations).

In this context, wastewater treatment in cities, industries and farms aims at reducing water pollution generated by anthropogenic activity. Wastewaters are mainly classified depending on their origin as domestic, industrial and agro-industrial (Chen et al., 2008). Depending on their origin, wastewaters exhibit a different physical, chemical and microbiological composition (Aditya et al., 2022). Domestic wastewaters are generated from human activities in households (e.g. use of the toilet, bathing, kitchen and washing), and therefore its production directly increases with human population. This type of wastewaters are characterized by low concentration of organic matter and nutrients due to the large water volume use in households (Table 1) (Hülsen et al., 2014). On the other hand, industrial wastewaters are one of the most diverse sources of wastewaters and their compositions are industry specific. For instance, brewery wastewaters are mainly composed of a high concentration of carbon (sugars and alcohols), while wastewater generated by the mining industry contains highly toxic and recalcitrant pollutants such as heavy metals (Table 1). Finally, agro-industrial wastewaters are produced by intensive agriculture, animal husbandry and their processing. Of them, wastewaters from husbandry of cows, poultry, pigs, turkeys and fishes rank among the most pernicious as a result of their large volumes of production and the gradual increase in animal production. Livestock wastewaters are characterized by a high content of organic matter from animal manure and a high content of ammonium from animal urine (Table 1).

Table 1. Characteristic composition in domestic, industrial an agro-industrial wastewater.

Composition	Domestic	Industrial[a]	Agro-industrial[b]
COD (mg L^{-1})	526	10000	54000
TN (mg L^{-1})	46	1	5000
TP (mg L^{-1})	6	3	1500
pH	6.8	7.0	7.7

[a] Industrial wastewater represented by brewery wastewater (Lu et al., 2013).

[b] Agro-industrial wastewater represented by piggery wastewater (Godos et al., 2009).

1.2. *Piggery wastewater*

The increased livestock farming for human food production represents a big environmental problem in regions such as Europe, Asia and North America, where the highest densities of animal farms are located (Fig. 2). For instance, the production of pigs in the European Union (EU) reached 23,720 thousand metric tons of pigs in 2021, with Spain as the second largest pork producer in the EU (Jiménez-Ruiz et al., 2022). Other countries such as China and United States produced 47,500 and 12,559 thousand metric tons of pigs in 2021, respectively (US Department of Agriculture). Intensive animal husbandry generates large volumes of wastewaters with high concentrations of organic matter, nitrogen and phosphorous, which requires the decentralized implementation of cost-effective wastewater treatment. Indeed, the uncontrolled discharge of pig manure entails a high impact in the environment due to the massive greenhouse gas emissions from anaerobic fermentation of pig manure and the released nitrogen and phosphorous, which negatively impacts biodiversity (Gilbert et al., 2018; Godos et al., 2010, 2009; Hülsen et al., 2022). Piggery wastewater (PWW) is considered one the most polluting agro-industrial wastewaters (Godos et al., 2009), characterized by high concentrations of organic matter (in form of volatile fatty acids), nitrogen (mainly in the form of ammonium) and even presence of heavy metals (such as copper, lead and zinc) (Chen et al., 2018; Godos et al., 2009). In addition, the inadequate management of this wastewater can cause malodor pollution in the surrounding areas.

Depending on the farming practices and type of pig farmed (litters, piglet or sows), PWW concentration can vary due to differences in animal nutrition, farming practices and water usage in the cage washing and maintenance (Table 2). Overall, these wastewaters are characterized by their high concentration of dissolved carbon, nitrogen and phosphorus, and suspended solids.

1.3. *Piggery wastewater treatment technologies*

Multiple technologies have been proposed and investigated for the treatment and valorisation of PWW. Traditionally, PWW has been treated mainly by aerobic or anaerobic processes, and even by a combination of thereof. One the most commonly im-

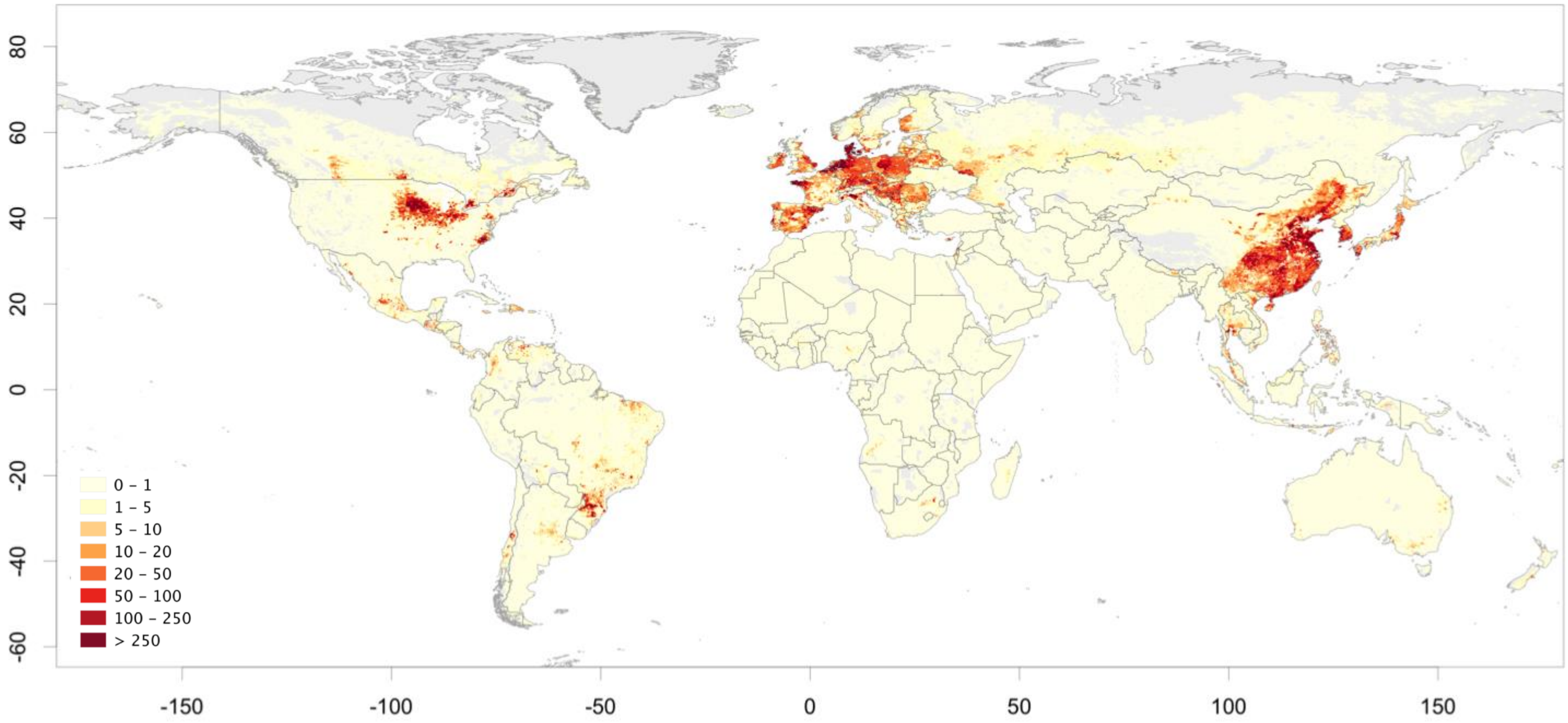

Figure 2. Distribution of intensive pigs production density in the world (pig head per square kilometre). Gray and green areas are considered unsuitable and protected areas (adapted from Gilbert et al., 2018, 2015).

Table 2. Pollutant concentrations in PWW from different farm around the world. Carbon is estimated as COD concentration.

Farm	Carbon (mg L^{-1})	Nitrogen (mg L^{-1})	Phosphorus (g L^{-1})	TSS (mg L^{-1})	Reference
Daejeon (South Korea)	18700	810	290	9120	(Myung et al., 2004)
Barcelona (Spain)	7450	785	120	3100	(Obaja et al., 2005)
Yokohama (Japan)	5300	1270	-	5900	(Joo et al., 2006)
Castilla y León (Spain)	54000	5000	1500	-	(Godos et al., 2009)
Seosan-si (South Korea)	8420	1150	34	-	(Lee et al., 2021)
Santiago (Chile)	18400	1140	2104	-	(Palominos et al., 2021)
Minas Gerais (Brazil)	104	90	20	-	(Silveira et al., 2021)
Queensland (Australia)	4130	1160	160	2713	(Hülsen et al., 2022)

plemented approaches for PWW management is the use of single-stage or multi-stage anaerobic lagoons to store and treat PWW (Montefiore et al., 2022). This approach is responsible for the release to the open atmosphere of high concentrations of greenhouse gases such as CO_2, CH_4, NH_3 and H_2S, with several environmental adverse effects (Fig. 3). The decanted manure from these anaerobic lagoons is typically used as a fertilizer due to its high concentration of nitrogen and phosphorous, despite it might contain antibiotics and heavy metals that accumulate and build-up in the food chain. In addition, these effluents contain a significant concentration of pathogens that are released in areas where manure is spread, thus polluting surface water and groundwater (Campagnolo et al., 2002).

Figure 3. Piggery farm and anaerobic lagoon in North Carolina of United States (adapted from Montefiore et al. (2022)).

Another conventional technology for PWW treatment is anaerobic digestion. This process is carried out by the symbiotic action of bacteria and archaea in the absence of O_2 or oxidized forms of nitrogen. Those microorganisms degrade the organic matter present in PWW producing mainly methane (CH_4) at concentrations ranging from 50 to 75%, carbon dioxide (CO_2) at concentrations of 25 to 45% and other gases at lower concentrations such as hydrogen (H_2 > 1%), ammonium (NH_3 > 1%), oxygen (O_2 > 2%) and hydrogen sulphide (H_2S > 1%) (Manyi-Loh et al., 2013). This type of technology allows the conversion of PWW into a renewable gas energy product, namely biogas. However, anaerobic digestion is only capable of removing the carbon present in PWW, generating liquid effluents with high concentrations of dissolved nitrogen and phosphorus, which are not assimilated by the anaerobic microbial community during this process. Additionally, this process generates solid sludge corresponding mainly to the non-biodegradable fraction of PWW and the anaerobic biomass formed in the process, which must be disposed of in landfills prior composting.

Activated sludge treatment is an aerobic process that is also typically used for PWW treatment despite its high energy demand (Montes et al., 2015). This process consists of an initial removal of suspended solids followed by an aerobic bioreactor with activated sludge (Fig. 4), and a final step of settling to collect the biomass generated (Suzuki et al., 2010). The aerobic tank, where carbon is oxidized to CO_2 and H_2O, and ammonium to nitrate or nitrate, can be preceded by an anoxic tank devoted to heterotrophic denitrification ($NO_3 \rightarrow N_2$). Activated sludge systems are dominated by bacteria, although eukaryotes (protozoa and fungi), archaea and viruses are typically found in this type of process. This process is the most studied and extensively implemented worldwide for the treatment of domestic wastewaters. Unfortunately, this process needs air supplementation via mechanical aeration for an adequate oxygenation by means of blowers or turbines, which involves high operating costs due to the high energy demand associated. In addition, another limitation of activated sludge processes derives from the fact that the removal of carbon and nitrogen is carried out mainly via volatilization into the atmosphere. Indeed, the organic matter present in PWW is converted into CO_2, which contributes to the production of greenhouse gases, while nitrogen is converted into N_2 through nitrification and denitrification processes or volatilize as NH_3. Finally, the heavy metals such as copper and zinc present in PWW are retained in the biomass generated, which is discarded in landfills.

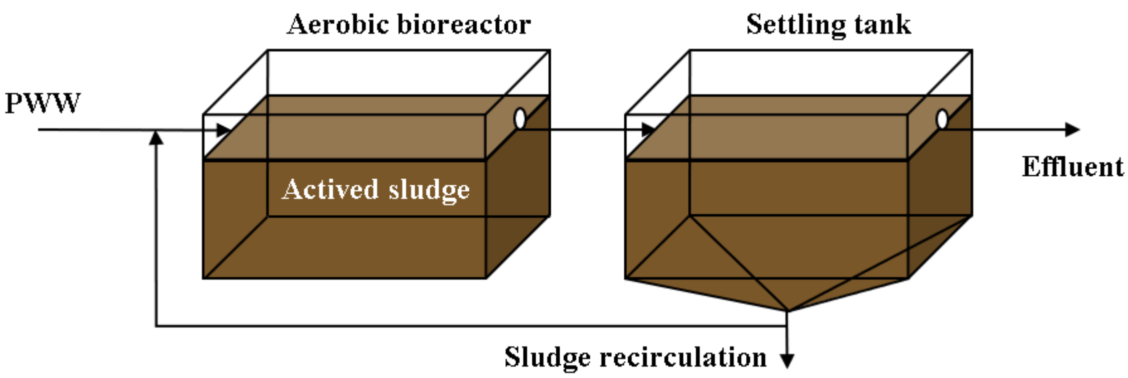

Figure 4. Schematic diagram of a conventional activated sludge process for PWW treatment. The system is composed of an activated sludge bioreactor interconnected to a settling tank.

1.4. *Photosynthetic piggery wastewater treatment*

The high environmental impacts, and high operating and energy costs of conventional anaerobic and aerobic treatments used for PWW treatment, have promoted research on photosynthetic PWW treatment in recent years (García et al., 2019; Godos et al., 2010, 2009; Hülsen et al., 2022, 2018b; Marín et al., 2019). Photosynthetic microorganisms are mainly classified in two groups according to their unique metabolism. The first group is composed of purple phototrophic bacteria (PPB), microorganisms that perform anoxygenic photosynthesis (without oxygen production) (Fig. 5). This group is subdivided into two bacterial group: purple sulphur bacteria (PSB) and purple non-sulphur bacteria (PNSB) (Capson-Tojo et al., 2020). PPB were originally classified based on their sulfide tolerance and characteristic purple-red colour, although PPB were recently classified according to its class within the phylum *Proteobacteria*, where PSB belong to the class *Gammaproteobacteria* and PNSB belong to the class *Alphaproteobacteria* or *Betaproteobacteria* (Hunter et al., 2009). Another photosynthetic bacterial group capable of performing anoxygenic photosynthesis are green sulphur bacteria (GSB) and green non-sulphur bacteria (GNSB), classified in these group according to their sulphur tolerance and their characteristic green colour.

On the other hand, microalgae are a diverse group of photosynthetic microorganisms with eukaryotic and prokaryotic species living in marine, freshwater and terrestrial habitats capable of performing oxygenic photosynthesis (oxygen production). The major classification of eukaryotic microalgae is subdivided in *Chlorophyta*, *Rhodophyta* and *Phaeophyta*, which were originally described according to their characteristic colour (green, red and brown, respectively) (Chan et al., 2022). Another group of eukaryotic microalgae are diatoms, which are characterized by cell walls composed of two siliceous pieces and classified into three classes: *Coscinodiscophyceae*, *Mediophyceae* and *Bacillariophyceae* (Medlin, 2016). In addition, prokaryotic microalgae belonging to the phylum *Cyanobacteria* have evolved in a wide variety of freshwater and saltwater environments.

PPB and microalgae possess extraordinary metabolic capabilities, and are able to grow at low temperatures or extremely high temperatures, in different pH ranges (alkaline or acidic), at high salt concentrations and even under the presence or absence of oxygen (Hallenbeck, 2017; Hunter et al., 2009; Madigan et al., 2021).

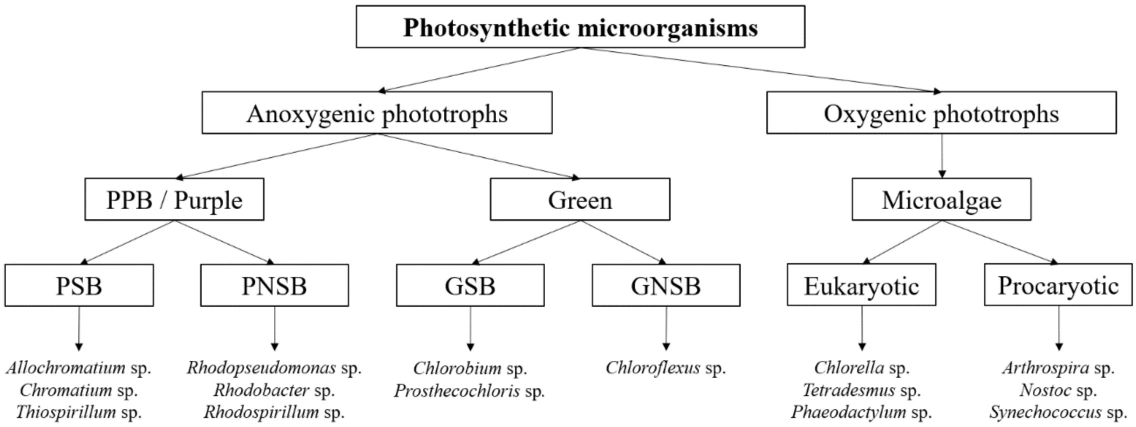

Figure 5. Diagram of photosynthetic microorganisms classification performing anoxygenic or oxygenic photosynthesis.

1.4.1. *Purple phototrophic bacteria-based treatment*

Purple phototrophic bacteria are gram-negative bacteria, prokaryotes and have the most versatile metabolism among microorganisms (Fig. 6). *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Rhodospirillum rubrum* from the PNSB group, and *Allochromatium vinosum* and *Thiocapsa roseopersicina* corresponding from the PSB group ranked among the most investigated PPB (Hunter et al., 2009; Puyol et al., 2020). PPB are capable of growing under anaerobic and aerobic conditions via phototrophic and chemotrophic metabolisms, respectively (Larimer et al., 2004). In addition, PPB are capable of assimilating and growing using organic or inorganic compounds as a carbon source under heterotrophic and autotrophic metabolism, respectively. Some PPB species can obtain energy for growth from the oxidation of inorganic compounds chemolithotrophically using H_2 or $S_2O_3^{-2}$ as electron donors (Hunter et al., 2009). PPB can grow in environments with extreme conditions: high concentrations of salt (Hülsen et al., 2019), extremely high temperatures or low temperatures in the range of 10-11 ºC (Dalaei et al., 2019; Hülsen et al., 2016a) and very acid or alkaline conditions (Hallenbeck, 2017).

PPB can obtain energy though of anoxygenic photosynthesis process, absorbing energy from the sunlight radiation reaching the earth surface by photon absorption using photosynthetic complexes. In this process, PPB produce ATP via photophosphorylation under phototrophic growth (Capson-Tojo et al., 2020; Hunter et al., 2009). Indeed, PPB

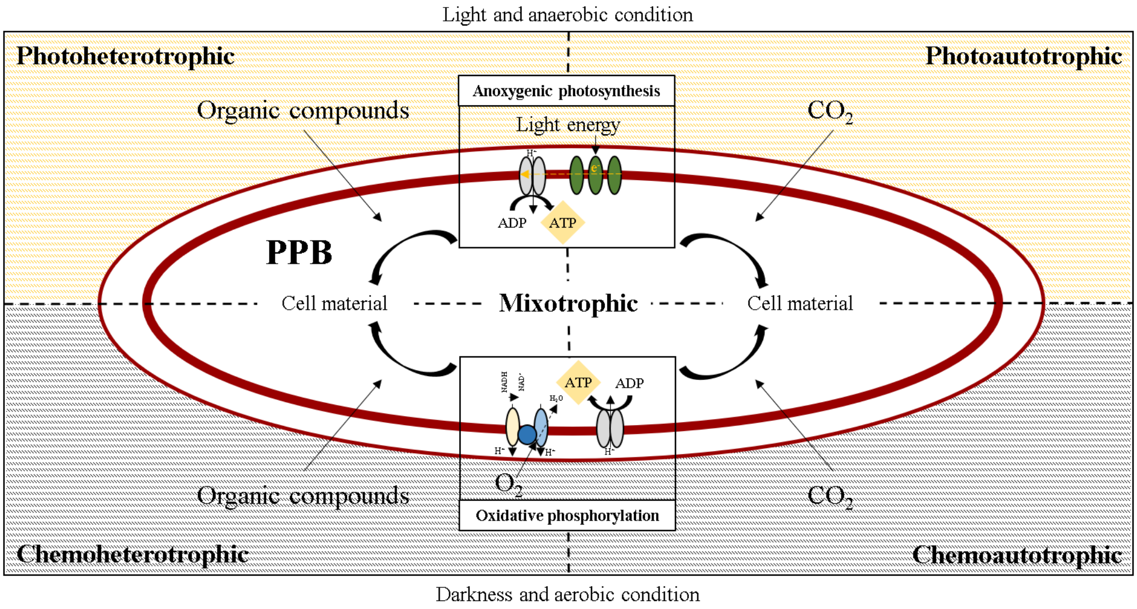

Figure 6. Metabolic diagram of purple phototrophic bacteria based on *Rhodopseudomonas palustris* (Méndez et al., 2022).

are more efficient photosynthetic microorganisms in the photoconversion compared to microalgae (Hülsen et al., 2014). The first descriptions of the basic mechanisms underlying photosynthesis were based on investigation using PPB as model microorganisms (Cogdell et al., 1999). Electron flow during the photosynthetic process in PPB begins with photons absorption by multiple light-harvesting (LH) complexes composed of pigments (mainly of bacteriochlorophylls and carotenoids) bonded to proteins. The absorbed energy is conducted to the reaction centre (P870), where photosynthetic electron transport reactions begin. When P870 is excited (representation P870*, Fig. 8), it becomes a very strong electron donor, capable of transferring energize electrons to a quinone (Q) located within the cytoplasmic membrane. These electrons, in turn, are donated to the cytochrome bc_1, which translocates four protons for every two electrons from the reaction centre. This process entails an increase in the proton concentration in the periplasm of PPB, which triggers the proton motive force to the cytoplasm, where ATPase enzyme synthetizes adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate. In contrast to oxygenic photosynthesis (a process performed by microalgae), the electrons from cytochrome in PPB are not transferred to an electron acceptor (such as molecular oxygen) but to cytochrome C2, and return to the reaction centre P870, closing the cyclic photophosporylation in PPB (Madigan et al., 2021).

PPB are a unique group of microorganisms capable of absorbing radiation of wavelengths in the near-infrared (NIR) spectrum (Fig. 7). Indeed, PPB exhibit a special spectral niche among photosynthetic microorganisms due to the presence of bacteriochlorophyll *a* and *b*, which present a maximum absorption at 804 nm and 1040 nm, respectively (Stomp et al., 2007). PPB contain carotenoids that absorb radiation below 500 nm and provide its characteristic purple-red colour. In addition, carotenoids are able to reduce the photodegradation of bacteriochlorophyll under high irradiance conditions.

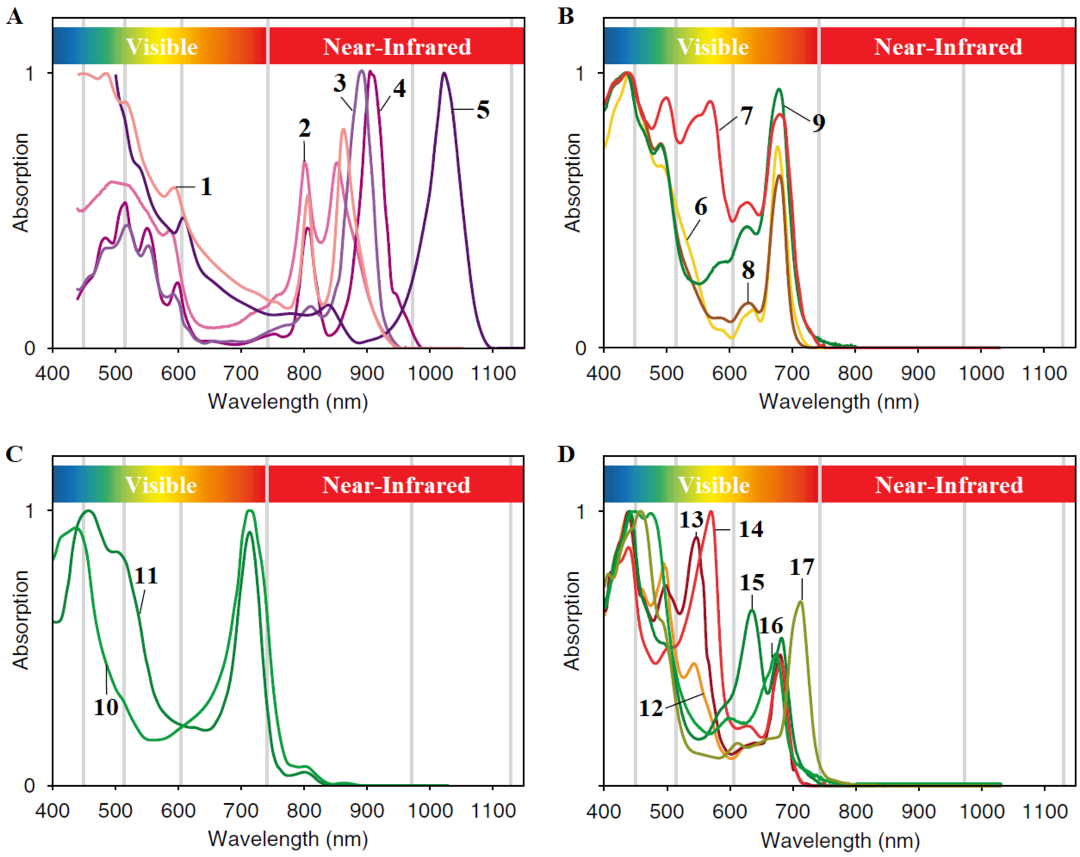

Figure 7. Absorption spectrum (400-1150 nm) of the purple phototrophic bacteria (A) *Rhodobacter capsulatus* (1), *Rhodobacter sphaeroides* (2), *Rhodospirillum rubrum* (3), *Roseospirillum parvum* (4), *Blastochloris viridis* (5), the microalgae (B) *Phaeodactylum tricornutum* (6), *Palmaria palmata* (7), *Isochrysis* sp. (8), *Chlamydomonas* sp. (9), the green sulphur bacteria (C) *Prosthecochloris aestuarii* (10), *Pelodictyon phaeoclathratiforme* (11) and the cyanobacteria (D) *Synechococcus* WH7803 (12), *Synechococcus* WH8103 (13), *Synechococcus* BS5 (14), Synechococcus BS4 (15),

Prochlorococcus sp. (16) and *Acaryochloris marina* (17) (adapted from Stomp et al. (2007)).

On the other hand, PPB can grow aerobically under chemoheterotrophic or chemoautotrophic metabolisms, obtaining energy via oxidative phosphorylation (Fig. 6) by degrading macromolecules for ATP synthesis (Lu et al., 2011b, 2011a). Molecular oxygen is used an electron acceptor under these growth conditions. However, this O_2 causes a repression and inhibition of bacteriochlorophylls synthesis, which harms the photosynthetic capacity of PPB (Izu et al., 2001).

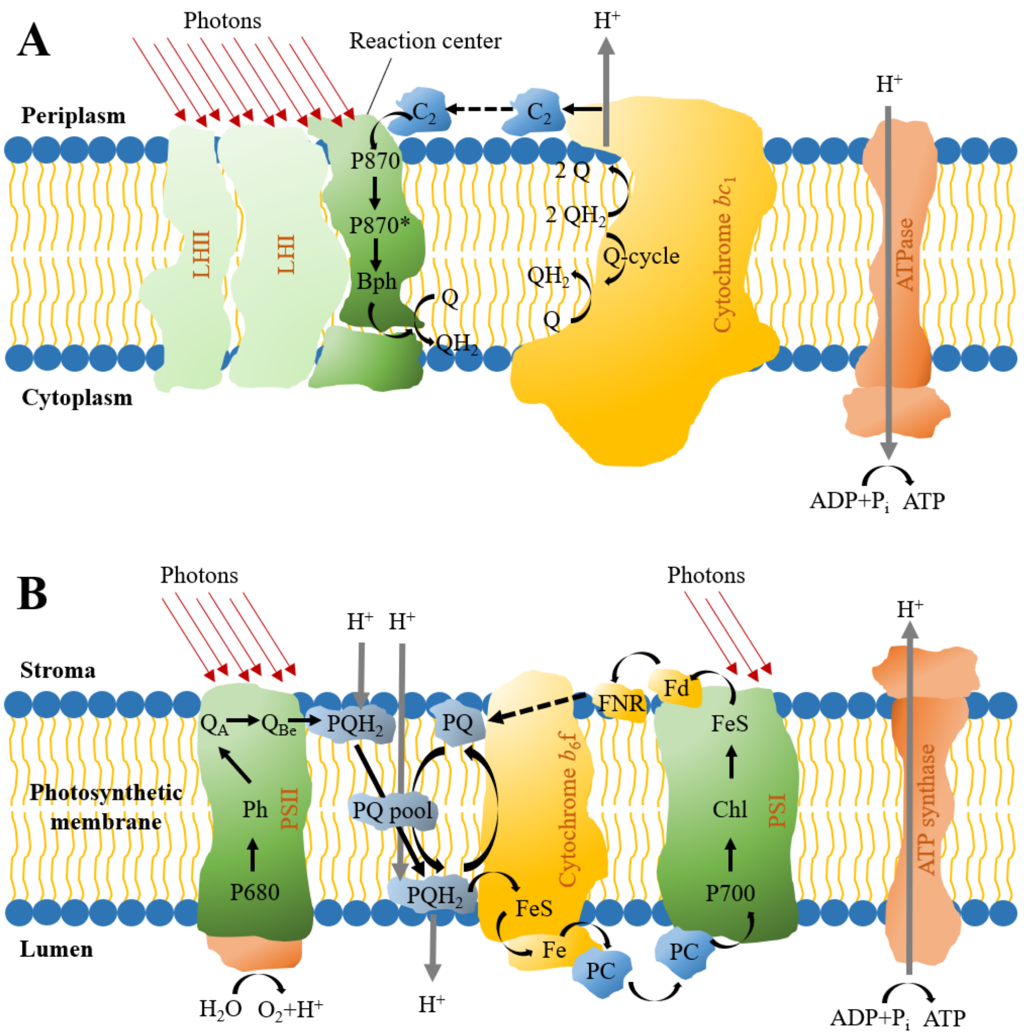

Figure 8. Diagram of electron transport (black lines) in anoxygenic (A) and oxygenic (B) photosynthesis in photosynthetic microorganisms. Light-harvesting (LH), bacteriochlorophyll (Bph), quinone (Q), cytochrome C_2 (C_2), photosystem (PS), plastoquinone (PQ), plastocyanin (PC), chlorophyll (Chl), ferredoxin (Fd), NADP oxidoreductase (FNR) (adapted from Madigan et al., 2021).

R. palustris is a PPB that holds multiple metabolic pathways like the tricarboxylic acid cycle, Embden-Meyerhof pathway, pentose phosphate route, fatty acids metabolism and even can fix CO_2 via the Calvin-Benson-Bassham (CBB) cycle (Larimer et al., 2004), which confer this microorganism an efficient metabolism for the assimilation of alcohols, amino acids, carbohydrates, fatty acids, organic acid and even recalcitrant pollutants in wastewater (Capson-Tojo et al., 2020). It has been recently described that *R. palustris* can re-assimilate the carbon produced in the catabolic pathways and strive for maximum carbon efficiency as most CO_2 produced from acetate oxidation is fixed and used for biomass production (Navid et al., 2019). In addition, PPB can assimilate all forms inorganic of nitrogen such as NO_3^-, NO_2^-, N_2 and NH_4^+ (Lu et al., 2019). This ability to fix N_2 is remarkable due to the high energy contained in the triple bond of this molecule. In this context, PPBs are able to synthesize the enzyme nitrogenase, which breaks the triple bond present in the N_2 and generates NH_3 using the energy contained in ATP. PPB can also assimilate the organic nitrogen present in amino acids such as aspartate, glutamate and glutamine. Certain species of PPB can even assimilate aromatic compounds like cyclohexane carboxylate, toluene, benzoate and their derivatives (Hunter et al., 2009). These versatile metabolic pathways provide PPB a great potential for the effective treatment of different types of wastewaters.

PPB can treat a wide variety of wastewaters from domestic, industrial and agro-industrial sources with high pollutants removal efficiencies (Capson-Tojo et al., 2020; Lu et al., 2019). For instance, concomitant removal efficiencies of 63% for COD, 99% for NH_4-N and 88% PO_4-P have been recorded during domestic wastewater treatment using PPB in batch tests with acetate supplementation (Hülsen et al., 2014). In addition, domestic wastewater treatment by PPB under low radiation intensities (< 3 W m^{-2}) with high removal efficiencies over 90% for COD and up to 86% and 91% for TN and TP removal, respectively, has been reported in literature (Dalaei et al., 2020). In addition, an effective domestic wastewater was achieved in a novel continuous photoanaerobic membrane bioreactor with a PPB dominance in the culture broth over 60% (Hülsen et al., 2016b). Similarly, high COD removal efficiencies have been reported during industrial wastewater treatments with PPBs: COD removal of 96% in brewery wastewater (Yang et al., 2018), 90% in acidic food wastewater (Liu et al., 2016) and 89% in VFA-rich food industry wastewater (Liu et al., 2019). On the other hand, PPB can also support satisfactory organic matter and nutrient removals during agro-industrial wastewaters treatment. For instance, PPB-based treatment provided removal efficiencies higher than 90% for COD and TN during poultry processing wastewater treatment (Hülsen et al., 2018b), > 83% for COD during digested piggery wastewater treatment (Wen et al., 2016) > 87%, 83% and 89% for TOC, TN and TP removal during PWW treatment (García et al., 2019), > 78% for TOC during piggery wastewater treatment coupled with biogas upgrading (Marín et al., 2019).

1.4.2. *Microalgae-based treatment*

Microalgae are photosynthetic microorganisms similar to plants on the fact that they carry out the oxygenic photosynthesis process and both present similar pigments belonging to chlorophyll and carotenoids group. Microalgae are the main photosynthetic microorganisms studied for wastewater treatment since the pioneer investigations of Oswald (1988). In addition, in the past decades a huge research effort was devoted to microalgae-based technologies for biofuel production, wastewater treatment and production of high value-added bioproducts from microalgae biomass (Chisti, 2007; Christenson and Sims, 2011; Muñoz and Guieysse, 2006; Posadas et al., 2017). The main microalgae species investigated were *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum* and *Arthrospira platensis* among others. Microalgae can grow photoautotrophically fixing CO_2 or photoheterotrophally by aerobically metabolizing organic compounds. Both metabolisms utilize energy from sunlight as the main energy source. Microalgae use H_2O as electron donor during photoautotrophic growth to produce O_2 as a by-product of the reaction (Madigan et al., 2021). Indeed, this photosynthetic oxygen production is crucial to sustain life in the planet since microalgae produce massive amounts of oxygen in the vast oceans and surface freshwater bodies. This ability to produce oxygen has been successfully used for wastewater treatment, where microalgae grow in symbiosis with bacteria. These bacteria use oxygen as electron acceptor for organic matter and ammonium oxidation, and are able to assimilate the ammonium and phosphorous present in wastewater. In addition, microalgae benefit from essential vitamins and CO_2 released by pollutant degrading bacteria (Fig. 9) (Godos et al., 2009; Nagarajan et al., 2022).

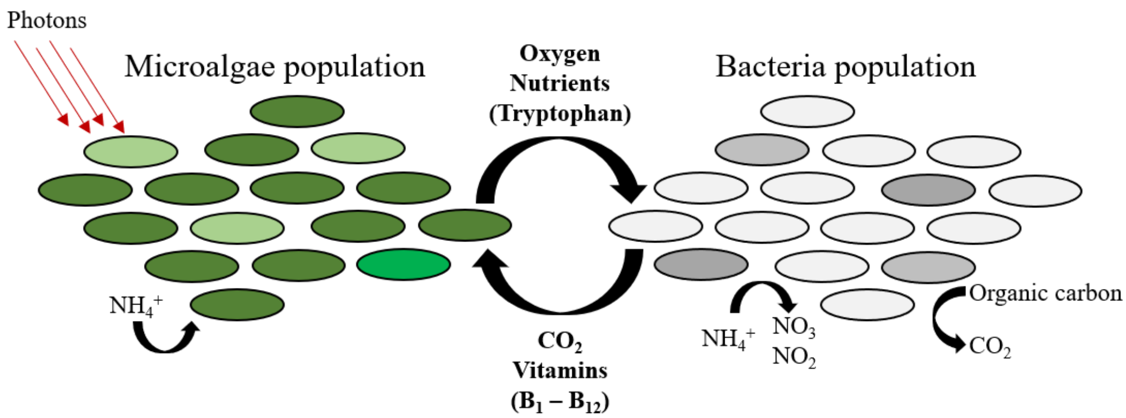

Figure 9. Metabolic interactions between microalgae and bacteria during wastewater treatment (adapted from Nagarajan et al. (2022)).

Photosynthesis in microalgae in comparison to PPB differs mainly on the fact that microalgae have two different photosystems for harvesting photons from solar irradiation, photosystem I (PSI or P700) and the photosystem II (PSII or P680). P680 catalysed the main step in oxygenic photosynthesis, where H_2O is split into O_2 and electrons (Fig. 8). This step starts when light energy is absorbed by P680 which donates an electron to pheophytin *a* (Ph), turning P680 strongly electropositive and with the capacity that accept electrons from H_2O. The oxidation of H_2O is produced in a water-oxidizing complex catalysed by a Mn_4Ca cluster. Electrons are transported through different quinones (Q_A and Q_B) within P680. Those electrons are used to reduce plastoquinone (PQ) to PQH_2. Electrons from PQH_2 are transferred to cytochrome b6f. From cytochrome b_6f, electrons are transferred to P700 through a copper-containing plastocyanin (PC). The absorption of photons by P700 allows to accept electrons from PC. These different steps generate a proton motive force that is used by ATP synthase to produce ATP. This photosynthesis process is noncyclic since the electrons do not return to P680 and are used for the generation of NADPH from $NADP^+$. Nevertheless, electrons can return from PSI to PSII in some species of cyanobacteria via an electron transport chain linked (dashed line in Fig. 8) (Madigan et al., 2021).

The main pigments present in the photosystems of microalgae are chlorophylls, which provide them with their characteristic green colour. Chlorophyll *a* exhibits a maximum absorption at 430 nm and 680 nm, while chlorophyll *b* absorbs mainly at 450 nm and 640 nm (Madigan et al., 2021; Stomp et al., 2007). In addition, microalgae present a variety of carotenoids such as astaxanthin, β-carotene and lutein that absorb mainly at wavelengths between 400 nm and 520 nm (Fig. 7). Carotenoids mainly act as accessory pigments in photon absorption. However, they also have a photoprotective function, preventing damage to the photosystems as a result of microalgae exposure to high irradiations (Hunter et al., 2009). On the other hand, cyanobacteria also contain accessory pigments belonging to the phycobiliprotein group, mainly phycocyanin, phycoerythrin and allophycocyanin. Phycocyanin and allophycocyanin are a blue pigments that absorb mainly at 620 nm and 650 nm (Aditya et al., 2022). Phycoerythrin is a red pigment that absorbs mainly in the range of 550 nm. Overall, most pigments present in microalgae and cyanobacteria absorb radiation in the visible spectrum (Fig. 7).

Microalgae have a powerful photoautotrophic metabolism that allow them to grow using solar energy and assimilating inorganic compounds. Microalgae can efficiently fix CO_2 via the Calvin cycle as the main carbon source, although are also capable of using $NaHCO_3$ or Na_2CO_3 under photoautotrophic mode. The preferred forms of nitrogen used by microalgae under photoautotrophic mode are ammonium (NH_4^+) and nitrate (NO_3^-) (Silveira et al., 2021), which are used for the synthesis of amino acids and proteins. Some cyanobacteria species are able to fix molecular nitrogen (N_2) from the atmosphere as the main nitrogen source. On the other hand, microalgae are able to grow photoheterotrophically using organic compounds, such as carboxylic acid, glucose and glycerol, as a carbon source, and amino acids, peptone and urea as a nitrogen source (Perez-Garcia et al., 2011). However, PPB are more efficient than microalgae under photoheterotrophic growth and biomass production photoheterotrophically is more expensive when synthetic organic compounds are used.

A part from nitrogen assimilation, the symbiotic association between microalgae and bacteria (Fig. 9) can support a sequential nitrification and denitrification, which can further boost nitrogen removal during domestic and agro-industrial wastewater treatment. The nitrification process is performed by ammonia oxidizing and nitrite oxidizing bacteria which can transform ammonium (NH_4^+) into nitrite (NO_2^-) and nitrite into nitrate (NO_3^-), respectively (Chai et al., 2021). Heterotrophic denitrification is an anoxic process performed by heterotrophic bacteria that converts NO_3^- to NO_2^- and finally to nitrogen gas (N_2).

Microalgae in association with bacteria represent the most widely studied group of photosynthetic microorganisms for wastewater treatment. In this context, an efficient treatment of domestic wastewater with TOC removals of 88% and TN of 82% was recorded in anoxic-aerobic photobioreactors (García et al., 2017), while the treatment of textile wastewater using this innovative configuration achieved TOC removals of 48%, TN removals of 87% and TP removals 57%, along with an efficient decolourization of this wastewater (Dhaouefi et al., 2018). An efficient treatment of synthetic food waste digestate, with removal efficiencies of up to 96% and 84% for TOC and TN, respectively, was recently reported by Torres-Franco et al. (2021). In addition, multiple studies of agro-industrial wastewater treatment reporting an efficient carbon and nitrogen removal have been reported for PWW (García et al., 2019; Godos et al., 2010, 2009; Marín et al., 2019).

1.5. *Photobioreactors for wastewater treatment*

The photobioreactors used for the cultivation of photosynthetic microorganisms are typically classified according to their contact with the atmosphere in open or closed photobioreactors. Photobioreactor design criteria involves a high irradiated surface area, effective mixing and good scalability (Muñoz and Guieysse, 2006). Multiple shapes and configurations have been described and investigated for the design of these photobioreactors. In this context, the most common configurations for PPB and microalgae culture are open ponds, open raceways, closed tubular and photo-anaerobic membrane bioreactor (Fig. 10) (Méndez et al., 2022). Open configurations exhibit low investment and operational costs compared to closed photobioreactors. However, they are susceptible to contamination by unwanted microorganisms, the conditions inside the photobioreactor are difficult to control and they have high-water losses by evaporation due to their open configuration. On the other hand, the main disadvantage of closed photobioreactors is their high investment costs. Nevertheless, the limited contact with the open atmosphere avoids microbial contamination of the culture broth, enhances the control of physical-chemical parameters and minimizes water losses.

However, both open and closed photobioreactors must maintain appropriate physical-chemical conditions to support an efficient growth of photosynthetic microorganisms. The key environmental parameter for the cultivation of these microorganisms is radiation, which is necessary for the photosynthetic process but requires extensive surface areas for an effective collection. On the other hand, an optimal control of temperature, pH and dissolved oxygen inside photobioreactors favours PPB and microalgae growth.

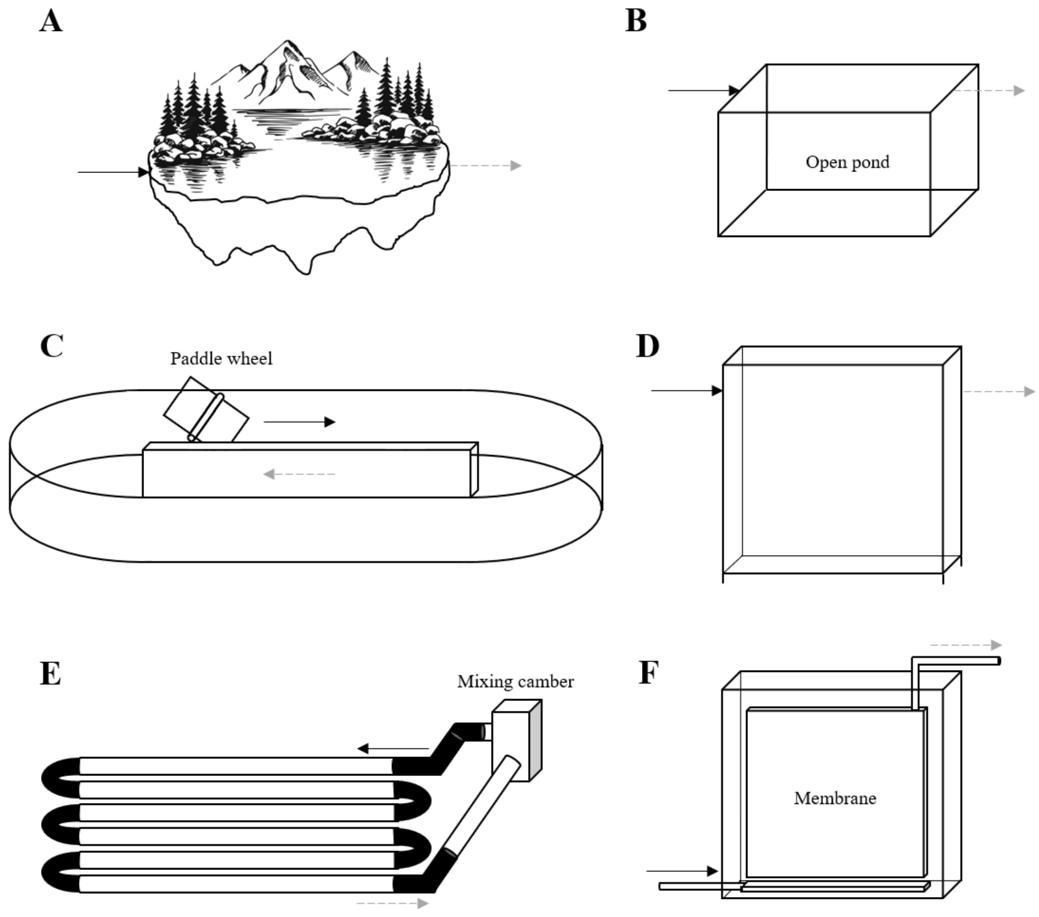

Figure 10. Schematic diagram of common photobioreactors for photosynthetic microorganisms cultivation. Natural lake (A), open pond (B), open raceways (C), vertical flat-panel (D), closed tubular (E) and photo-anaerobic membrane bioreactor (F).

Light intensity is the main parameter for the cultivation of photosynthetic microorganisms via phototrophy. The electromagnetic spectrum of solar light provides the energy source for the photosynthetic process. In this regard, photosynthetically active radiation (PAR) is the wavelength range suitable to support the growth of photosynthetic organisms (mainly plants and microalgae), corresponding to wavelengths between 400 nm and 780 nm (visible spectrum), where light absorption by chlorophylls and carotenoids occurs (Fig. 7). On the other hand, PPB can absorb radiation in both visible and near-infrared (NIR) spectrum (Fig. 11). At high radiation intensities and low culture densities, inhibition of photosynthetic activity can occur, resulting in an increase in intracellular carotenoid concentrations or inhibiting growth totally. However, process operation with dense microalgae cultures can be sustained under light intensities of up to 1300 µmol m^{-2} s^{-1}, which represents the average solar intensity on a clear day (García et al., 2019; Marín et al., 2019).

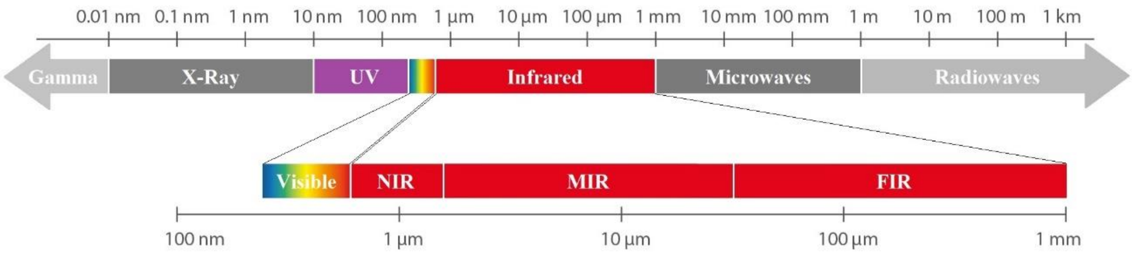

Figure 11. The electromagnetic spectrum, extension of visible spectrum (400-780 nm) and infrared spectrum (780-1000000 nm). Infrared spectrum is composed of near-infrared (NIR: 0.780-3000 nm), mid-infrared (MIR: 3-50 µm) and far-infrared (FIR: 50-1000 µm).

Temperature is also a key parameter for cultivation of photosynthetic microorganisms. Psychrophiles are microorganisms that grow in the temperature range between 0 to 15 °C, mesophiles in temperature range between 15 to 45 °C and thermophiles are microorganisms whose growth optimum temperature exceeds 45 °C (Madigan et al., 2021). PPB and microalgae species corresponding to these three groups have been consistently reported in literature. For instance, PPB species such as *Rhodoferax antarcticus* have been found in the Antarctic (Madigan et al., 2000), while the microalga *Chlamydomonas nivalis* is known to be responsible for the red colour in the snow surface (Madigan et al., 2021). In addition, an effective treatment of domestic wastewater at low temperatures (10-11 °C) using PPB has been observed (Dalaei et al., 2019; Hülsen et al., 2016a). Most common species of PPB and microalgae, such as *Rhodopseudomonas palustris* or *Chlorella vulgaris*, grow at moderated temperatures. Finally, PPB such as *Thermochromatium tepidum* and the cyanobacterium *Oscillatoria terebriformis* have been found in hot springs growing at temperatures above 55 °C (Hallenbeck, 2017). Most studies in literature were carried out using photosynthetic microorganisms at ambient temperatures between 20 to 30 °C.

Microorganisms can be classified in terms of their oxygen tolerance in three groups: i) aerobic microorganisms that can grow and tolerate high oxygen concentrations,

ii) microaerophiles that can grow in the presence of low concentrations of oxygen and iii) anaerobes that cannot grow in the presence of oxygen. The latter can be classified as strict or obligate anaerobes, when oxygen is a growth inhibitor that can even killing them, and oxygen tolerant, when oxygen has no biological function in their metabolism (Madigan et al., 2021). For instance, microaerophilic and anaerobic PPB can tolerate dissolved oxygen concentrations up to 1 mg L^{-1}, which are mainly used in the electron transport chain as an electron acceptor in the oxidative phosphorylation process (Lu et al., 2011a). However, oxygen can be harmful to PPB during wastewater treatment processes since the growth of aerobic chemotrophic bacteria is favoured (Capson-Tojo et al., 2021; Siefert et al., 1978). On the other hand, while microalgae can grow in the presence of oxygen, very high dissolved oxygen concentrations (> 25 mg L^{-1}) can cause damage in microalgae cells and inhibit their growth (Muñoz and Guieysse, 2006). Fortunately, the oxygen produced by microalgae is rapidly utilized (as electron acceptor) by bacteria for the oxidation of organic matter and NH_4 during wastewater treatment (Fig. 9).

1.5.1. *Open photobioreactors*

Open ponds were the oldest configurations proposed for photosynthetic microorganisms cultivation at industrial scale for microalgae biomass production (Carvalho et al., 2006). This configuration was proposed to mimic the most common growth of microalgae in nature, using natural or artificial lakes for microalgae culture with different depths. These ponds are less expensive to operate and build compared to other photobioreactor configurations at large-scale. However, their open configuration render them more sensitive to the variations in the weather conditions, to microbial contamination and exhibit high water evaporation levels (Mata et al., 2010). This first configuration was limited by the small photic zone when microalgae concentration is high and the high costs associated to nutrient homogenization.

The design of open ponds was improved in order to optimize microalgal activity by reducing their depth to favour photosynthetic activity and reducing energy costs for mixing, which resulted in the so called open raceways or high rate algal ponds. This type of photobioreactor consists of a shallow lagoon (deep of 0.2-0.4 m) with multiple channels and mixing via a paddle wheel motorised (Fig. 10) (Chisti, 2007). The continuous mixing by a paddle wheel favours the agitation of the culture broth, exposing the cells to constant radiation during the day (under solar radiation), which favours the photosynthetic process. This agitation mode is simple and requires a low electricity consumption. Numerous studies describing the cultivation of microalgae an even wastewater treatment in open raceways, with moderated-to-high microalgae productivities are available in literature (Chisti, 2007; Christenson and Sims, 2011; Marín et al., 2021, 2018; Muñoz and Guieysse, 2006; Posadas et al., 2017). On the other hand, PPB growth in 10 cm deep open raceways using aerobic PPB cultures (dominated by *Rhodobacter capsulatus*) in synthetic media has been reported (Alloul et al., 2021). The main disadvantage of open raceways and open photobioreactors are the high evaporation rate and the associated consumption of water to compensate these evaporation losses (Christenson and Sims, 2011).

1.5.2. *Closed photobioreactors*

Closed photobioreactors design involve a limited contact of the cultivation broth with the atmosphere. This configuration exhibits a superior performance in term of control of microorganisms growth, high surface area to volume ratio, low water evaporation losses, higher light availability and easy control of operational parameters, which increase photosynthetic biomass productivity compared to their open counterparts (Table 3) (Carvalho et al., 2006).

The most studied closed photobioreactors for cultivation of photosynthetic microorganisms are horizontal tubular and flat-panel photobioreactors due to their easier scalability. These photobioreactors need colourless material for their construction, such as glass, polymethyl-methacrylate or polyvinyl chloride (PVC), which increases their investment cost (Muñoz and Guieysse, 2006). In this sense, flat-panels photobioreactors using bags of colourless high-density polyethylene supported in a metal cage have been proposed as one of the most economical enclosed photobioreactors for the cultivation of microalgae. On the other hand, tubular configurations are easily scalable by increasing the number and length of tubes, and exhibit higher light utilization efficiencies compared to flat-panel photobioreactors. Both types of closed configurations favour the availability of solar radiation, enhance photosynthesis and reduce the footprint required for their construction.

Table 3. Principal advantages and disadvantages of open and closed configurations of photobioreactors used for the cultivation of photosynthetic microorganisms.

	Open photobioreactors	**Closed photobioreactors**	**Reference**
Capital investment	Low	High	(Davis et al., 2011)
Scalability	High	Variable	(Davis et al., 2011)
Culture control	Low	High	(Christenson and Sims, 2011)
Culture contamination	High	Low	(Christenson and Sims, 2011)
Evaporation rate	High	Low	(Christenson and Sims, 2011)
Biomass productivity	Variable	High	(Davis et al., 2011)
Nutrient removal	High	High	(Muñoz and Guieysse, 2006)
Nutrient recovery in biomass	Variable	High	(Davis et al., 2011)

In recent years, the most studied photobioreactor configuration for the cultivation of PPB is the photo-anaerobic membrane bioreactor (Hülsen et al., 2018b, 2016b, 2016a). This configuration is composed of a rectangular polymethyl-methacrylate photobioreactor with a submerged flat sheet membrane (0.45 µm pore size) and operated under anaerobic conditions (air-tight system) with internal gas recycling as agitation strategy (Hülsen et al., 2016b). This type of photobioreactor enables an excellent biomass concentration inside of the photobioreactor and provides an effluent with a low concentration of total solids. However, photo-anaerobic membrane bioreactors also present disadvantages such as the formation of biofilms on the internal walls of the photobioreactor and on the membrane, which hinders an adequate suspension of the PPB cultures. Additionally, the membrane module increases both the investment and operating cost of this technology.

1.6. *Biomass valorization*

The potential high productivities of photosynthetic microorganisms (PPB and microalgae) and the inherent presence of high value-added products represent a unique opportunity to turn wastewater treatment based on photosynthetic microorganism into a profitable process (Capson-Tojo et al., 2020; Chitapornpan et al., 2013). PPB biomass contains a high content of pigments (bacteriochlorophylls and carotenoids), single cell protein, coenzyme Q10, pantothenic acid, amino acid 5-ALA and polyhydroxyalkanoates (Lu et al., 2019). On the other hand, microalgae can synthetize high concentrations of carbohydrates, proteins, lipids and pigments (chlorophylls and carotenoids) (Ángeles et al., 2020). In addition, the biomass of photosynthetic microorganisms can be used as a feedstock for the production of biofuels like biodiesel and bioethanol (Brennan and Owende, 2010), while some species of PPB and cyanobacteria are capable of producing biohydrogen. PPB and microalgae biomass can be also used as a biofertilizer for plants as an N_2-fixing rhizosphere and are known to accumulate compounds that are beneficial for plant growth (Sakarika et al., 2019) (Fig. 12).

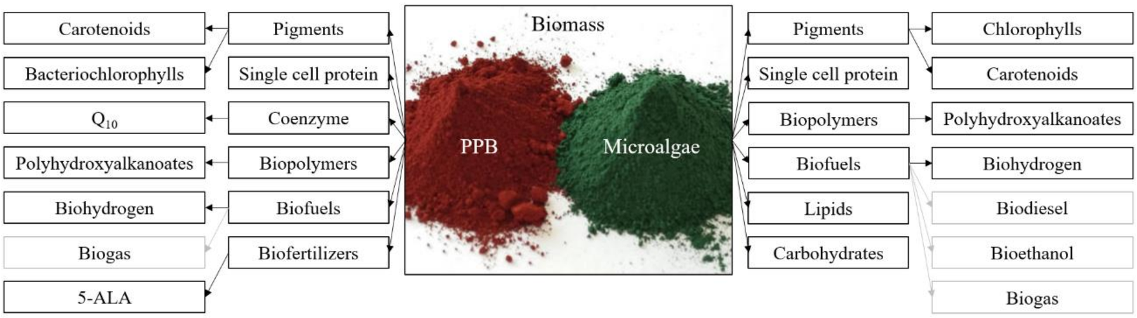

Figure 12. Summary of different potential products with high added value in photosynthetic microorganisms (PPB and microalgae). Gray frames require auxiliary processes.

The following sections summarize the main bioproduct potentially obtained from PPB and microalgae biomass.

1.6.1. *Pigments*

Photosynthetic microorganisms inherently present high concentrations and a broad portfolio of natural pigments since they are essential molecules for obtaining energy from solar radiation. PPB synthesize a variety of pigments mainly of the group of bacteriochlorophylls and carotenoids, which conform light harvesting complexes (Capson-Tojo et al., 2020). Bacteriochlorophyll *a* and *b* are pigments found mainly PPB, both pigments with a characteristic green colour. Carotenoids provide the characteristic orange to purple colours to the group of PPB (Hülsen et al., 2014). Thus, the major carotenoids synthesized by PPB are lycopene, rhodopsin, spheroidene, spirilloxanthin and their derivatives (Grassino et al., 2022; Hunter et al., 2009). On the other hand, microalgae are an important source of commercial high value compounds and can synthetize mostly pigments of the group of chlorophyll and carotenoids (Borowitzka, 2013). This last group of pigments include β-carotene, astaxanthin and lutein (Borowitzka, 2013; Lorenz and Cysewski, 2000). In addition, cyanobacteria can synthesize pigments belonging to the group of phycobiliproteins. An important and rare pigment of this last group is phycocyanin, a pigment with a striking blue colour that can absorb at wavelengths of ~620 nm and present promising antibacterial, anticancer, anti-inflammatory and antioxidant activities (Aditya et al., 2022; Wang et al., 2022). All those pigments are nowadays commercialized for the production of cosmetics, pharmaceuticals and functional foods.

1.6.2. *Single-cell protein*

Single-cell proteins, a term coined to refer to proteins from microbial origin, have been proposed as a sustainable substitute of traditional animal or vegetable proteins. Photosynthetic microorganisms can achieve a fast growth compared to plants and animals, and could potentially support high protein productivities, even when growing in low nutrient media or wastewater. This PPB and microalgae biomass valorization strategies is attracting and increasing attention due to the high commercial value of proteins for the production of animal feed or supplements for human nutrition (Capson-Tojo et al., 2020). PPB typically exhibits a protein content between 41% and 86% (Cao et al., 2020), while microalgae protein a content ranges between 28% and 71% (Spolaore et al., 2006). *Spirulina platensis* is the most cultivated microalgae (cyanobacteria) for commercial purposes due to its high content of protein (up to 70%) and essential amino acids supporting a high nutritional value. Indeed, *S. platensis* has been authorized for human consumption as a protein supplement (Mohammadi et al., 2022), and as a feed supplement in livestock and aquaculture (Lupatini et al., 2017). Single-cell protein can be produced independently of the climatic conditions and availability of arable lands, although nowadays their high costs of production limit the widespread commercialization of single cell protein. In this context, photosynthetic microorganisms can obtain the energy for growth from sunlight, use wastewater as a nutrient and water source and fix nutrient from atmosphere (CO_2 or N_2), which represents a unique combination to reduce the production costs of single-cell protein (Hülsen et al., 2018a).

1.6.3. *Coenzyme Q10*

PPB can synthesize coenzyme Q10 under phototrophic conditions and accumulate similar concentrations to other microorganisms such as *Escherichia coli* and *Rhizobium radiobater* (Capson-Tojo et al., 2020). The main function of this coenzyme in PPB is the transport of electrons in the electron transport chain, playing an essential role to produce ATP during oxidative phosphorylation. The coenzyme Q10 has antioxidant properties and is widely used in the cosmetic and medical industry (Zhu et al., 2017). Coenzyme Q10 supplementation in the skin can reduce the wrinkle formation. In addition, this compound prevents and causes beneficial effects in patients with diseases such as Parkinson, cardiovascular and hypertension, thus improving cellular bioenergetics and reducing the occurrence of free radicals due to its antioxidant properties (Kumar et al., 2009). These benefits for human health entail a high economical value of the coenzyme Q10, with current market prices of ~ €500 per kilogram and increasing demand worldwide in the last years (Capson-Tojo et al., 2020). This coenzyme can be efficiently produced in non-toxic wastewater treatment using PPB (He et al., 2021).

1.6.4. *Polyhydroxyalkanoates*

Polyhydroxyalkanoates are intracellular biopolymers accumulated by multiple photosynthetic microorganisms and bacteria as energy and carbon reserves (Fradinho et al., 2019). These molecules are a type of biopolymer with biodegradable properties and a high potential for substituting traditional pretrochemical plastics such as polypropylene or polyethylene (produced from petroleum derivatives), highly harmful to terrestrial and marine biodiversity (Chen and Patel, 2012). The production of polyhydroxyalkanoates in microorganisms using monocultures or mixed cultures requires a limitation of nitrogen or other essential nutrients to promote the accumulation of these biopolymers in PPB and microalgae (Capson-Tojo et al., 2020). Short feast periods or nutrient limitations favour the adaptation of microorganisms to accumulate polyhydroxyalkanoates over time (Fradinho et al., 2019). The production of polyhydroxyalkanoates from wastewaters by photosynthetic microorganisms requires low concentration of nutrients to favour the intracellular accumulation of this biomolecule under excess of carbon supply. The main PPB described in literature for their high polyhydroxyalkanoates accumulation capacity are *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* (Capson-Tojo et al., 2020). Likewise, cyanobacteria such as *Synechococcus* sp., *Nostoc muscorum* and *Spirulina platensis* have also been described as potential candidates to produce polyhydroxyalkanoates (Costa et al., 2019). Although more research is still needed to enhance the accumulation and production of polyhydroxyalkanoates in photosynthetic microorganisms, this solar driven technology platform represents an economic alternative to produce bioplastics.

1.6.5. *Biofuels*

Photosynthetic microorganisms can produce different types of biofuels, either directly synthesized such as biohydrogen or by additional processes utilizing their biomass such as biodiesel, bioethanol and biogas (Capson-Tojo et al., 2020). Biofuels represent a unique opportunity to boost the development of renewable energy technologies and to reduce CO_2 emissions from anthropogenic activity.

Photosynthetic microorganisms have emerged as potential candidates for biohydrogen production due to its high conversion efficiency and the broad portfolio of substrates used to sustain their growth. Biohydrogen (H_2) can be produced directly by PPB (*R. palustris, R. sphaeroides* and *R. rubrum*) mainly under a photoheterotrophic growth mode. Biohydrogen is a clean fuel since during its combustion (H_2 + O_2) only energy and water (H_2O) are produced (Adessi et al., 2012). This gas can be also used in fuel cells providing a very efficient alternative to combustion engines in transportation

(Fan et al., 2021). The production of H_2 in PPB occurs under illumination, anoxygenic conditions and nitrogen limitations because the synthesis of the enzyme responsible for hydrogen production (nitrogenase) is inhibited in the presence of ammonium (Adessi and De Philippis, 2014; Capson-Tojo et al., 2020). Likewise, microalgae and cyanobacteria species such as *Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *Synechocystis* sp. or *Anabaena* sp. can produce directly biohydrogen (Iqbal et al., 2022).

Biodiesel was proposed as a potential substitute for conventional petroleum-based fuels. Biodiesel can be produced from plant and animal oils or recently from microorganisms, with a special emphasis on the production from microalgae oil. Biodiesel is a mixture of fatty acid alkyl esters produced by a transesterification process (Arutselvan et al., 2022). This process includes three sequential steps, where triglycerides are converted in diglycerides, monoglycerides and finally converted into esters (biodiesel) and glycerol (by-product). The transesterification process involves a triglyceride and a short chain alcohol (e.g. methanol) in presence of a catalyst (e.g. sodium hydroxide) for synthesis. The triglyceride to biodiesel ratio is 1:1 (e.g. 100 g of biodiesel can be obtained from 100 g of triglycerides). The ability of PPB to produce transesterificable oils to produced biodiesel has not been yet investigated. However, the ability of microalgae to synthesize high lipid contents under nutrient limitation (40-75% for the species with the highest lipid production) has attracted a great interest and research efforts in the mass cultivation of this type of photosynthetic organisms to produce biofuel in the past 15 years (Chisti, 2007; Mata et al., 2010).

Bioethanol is a biofuel produced from the carbohydrates contained in plant biomass or microorganisms (Szulczyk and Tan, 2022). This biofuel is produced through alcoholic fermentation processes of hydrolysed biomass, which produce an ethanol with an identical chemical composition to that of chemical origin, and that also requires a distillation step for its purification (Brennan and Owende, 2010). Bioethanol can be used as a biofuel directly or mixed with conventional gasoline. In the past, ethanol production from microalgae biomass has been proposed based on its high carbohydrate content in terms of cellulose and starch, which can be fermented for bioethanol production (Ho et al., 2013). In addition, the CO_2 produced from ethanol combustion can be recovered since microalgae are capable of fixing CO_2, thus neutralising carbon emissions to the atmosphere and creating a cycling process for carbon utilization (Acebu et al., 2022;

Christenson and Sims, 2011).

Finally, the most studied biofuel produced from photosynthetic microorganisms biomass is methane. Methane can be produced from anaerobic digestion of PPB or microalgae biomass, where the degradation of the organic matter contain in these photosynthetic microorganisms occurs under anaerobic conditions in different steps catalysed by anaerobic bacteria and archaea (Chen et al., 2008). The first process in anaerobic digestion is a hydrolysis step, where complex organic matter is converted into simpler molecules such as amino acids, fatty acids and monosaccharides by different microorganisms. These simpler compounds are converted in a second acidogenesis step into volatile fatty acids and alcohols. The latter compounds are biotransformed by acetogenic bacteria during the acetogenic step into acetic acid, hydrogen and carbon dioxide as the main metabolites. Finally, methanogenic archaea transform the acetic acid, carbon dioxide and H_2 into biogas during the methanogenic step (Appels et al., 2008). Biogas is a mixture of gases mainly composed of high concentrations of methane (40-75%), carbon dioxide (15-60%), hydrogen sulfide (0.005-3%), nitrogen (0-2%) and oxygen (0-1%) (Ángeles et al., 2020). Biogas upgrading to biomethane is necessary to remove unwanted gases such as CO_2 and H_2S if biogas is used as vehicle fuel or injected into natural gas grids. This biogas upgrading step can be carried out using PPB (Marín et al., 2019) and microalgae (Muñoz et al., 2015; Xu et al., 2015; Zabed et al., 2020) based on the ability of these microorganisms to fix CO_2 and support H_2S oxidation.

1.6.6. *Biofertilizers*

Biofertilizers are fertilizer biological origin capable of promoting growth and the nutritional quality of plants. PPB can be directly used as biofertilizers (active benefit) since these photosynthetic microorganisms can grow in symbiosis with plants as rhizosphere and are capable to synthesize and excrete compounds promoting plant growth. For instance PPB can synthesize the amino acid 5-ALA, beneficial to plant growth (Lu et al., 2019). 5-ALA can be used as plant growth enhancer and even possesses insecticide and herbicide properties (Cao et al., 2020; Capson-Tojo et al., 2020). In addition, when PPB are associated with plant roots, they can contribute to heavy metal remediation in soils via accumulation into PPB biomass, which reduces adverse effects of these metals inside plant (Batool et al., 2017). In addition, PPB biomass can be used as dried biomass (passive benefit) due to its high nutrient (N and P) content (Capson-Tojo et al., 2020; Sakarika et al., 2019). PPB can accumulate phosphate in form of polyphosphates and fix nitrogen from the atmosphere, nutrients that are necessary for plant growth and limited in soils used for intensive agriculture (Sakarika et al., 2019). On the other hand, the merits of microalgae biomass as slow-release biofertilizers have been consistently reported in literature (Coppens et al., 2016). Microalgae biomass is also rich in nitrogen, phosphorous and trace elements such as potassium, calcium, magnesium and iron, that can be absorbed by plants when microalgal biomass is supplemented and even increase the nutritional quality of biofertilized fruits (Aditya et al., 2022).

1.7. *References*

Acebu, P.I.G., de Luna, M.D.G., Chen, C.Y., Abarca, R.R.M., Chen, J.H., Chang, J.S., 2022. Bioethanol production from Chlorella vulgaris ESP-31 grown in unsterilized swine wastewater. Bioresour. Technol. 352, 127086. https://doi.org/10.1016/j.biortech.2022.127086

Adessi, A., De Philippis, R., 2014. Photobioreactor design and illumination systems for H2 production with anoxygenic photosynthetic bacteria: A review. Int. J. Hydrogen Energy. https://doi.org/10.1016/ j.ijhydene.2013.12.084

Adessi, A., McKinlay, J.B., Harwood, C.S., De Philippis, R., 2012. A *Rhodopseudomonas palustris* nifA* mutant produces H2 from NH 4 + -containing vegetable wastes. Int. J. Hydrogen Energy 37, 15893–15900. https://doi.org/10.1016/j.ijhydene.2012.08.009

Aditya, L., Mahlia, T.M.I., Nguyen, L.N., Vu, H.P., Nghiem, L.D., 2022. Microalgae-bacteria consortium for wastewater treatment and biomass production. Sci. Total Environ. https://doi.org/10.1016/ j.scitotenv.2022.155871

Alloul, A., Cerruti, M., Adamczyk, D., Weissbrodt, D.G., Vlaeminck, S.E., 2021. Operational strategies to selectively produce purple bacteria for microbial protein in raceway reactors. Environ. Sci. Technol. 55, 8278–8286. https://doi.org/10.1021/acs.est.0c08204

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

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. https://doi.org/10.1016/j.pecs.2008.06.002

Arutselvan, C., Seenivasan, H. kumar, Lewis Oscar, F., Ramya, G., Thuy Lan Chi, N., Pugazhendhi, A., Thajuddin, N., 2022. Review on wastewater treatment by microalgae in different cultivation systems and its importance in biodiesel production. Fuel 324, 124623. https://doi.org/10.1016/j.fuel.2022. 124623

Batool, K., tuz Zahra, F., Rehman, Y., 2017. Arsenic-Redox Transformation and Plant Growth Promotion by Purple Nonsulfur Bacteria *Rhodopseudomonas palustris* CS2 and *Rhodopseudomonas faecalis* SS5. Biomed Res. Int. 2017, 1–8. https://doi.org/10.1155/2017/6250327

Borowitzka, M.A., 2013. High-value products from microalgae-their development and commercialisation. J. Appl. Phycol. https://doi.org/10.1007/s10811-013-9983-9

Brennan, L., Owende, P., 2010. Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2009.10.009

Cai, T., Park, S.Y., Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2012.11.030

Campagnolo, E.R., Johnson, K.R., Karpati, A., Rubin, C.S., Kolpin, D.W., Meyer, M.T., Esteban, J.E., Currier, R.W., Smith, K., Thu, K.M., McGeehin, M., 2002. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. Sci. Total Environ. 299, 89–95. https://doi.org/10.1016/S0048-9697(02)00233-4

Cao, K., Zhi, R., Zhang, G., 2020. Photosynthetic bacteria wastewater treatment with the production of value-added products: A review. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.122648

Capson-Tojo, G., Batstone, D.J., Grassino, M., Vlaeminck, S.E., Puyol, D., Verstraete, W., Kleerebezem, R., Oehmen, A., Ghimire, A., Pikaar, I., Lema, J.M., Hülsen, T., 2020. Purple phototrophic bacteria for resource recovery: Challenges and opportunities. Biotechnol. Adv. 43, 107567. https://doi.org/10.1016/j.biotechadv.2020.107567

Capson-Tojo, G., Lin, S., Batstone, D.J., Hülsen, T., 2021. Purple phototrophic bacteria are outcompeted by aerobic heterotrophs in the presence of oxygen. Water Res. 194, 116941. https://doi.org/10.1016/j.watres.2021.116941

Carvalho, A.P., Meireles, L.A., Malcata, F.X., 2006. Microalgal Reactors: A Review of Enclosed System Designs and Performances. Biotechnol. Prog. 22, 1490–1506. https://doi.org/10.1021/bp060065r

Chai, W.S., Chew, C.H., Munawaroh, H.S.H., Ashokkumar, V., Cheng, C.K., Park, Y.K., Show, P.L., 2021. Microalgae and ammonia: A review on inter-relationship. Fuel 303, 121303. https://doi.org/10.1016/j.fuel.2021.121303

Chan, S.S., Low, S.S., Chew, K.W., Ling, T.C., Rinklebe, J., Juan, J.C., Ng, E.P., Show, P.L., 2022. Prospects and environmental sustainability of phyconanotechnology: A review on algae-mediated metal nanoparticles synthesis and mechanism. Environ. Res. https://doi.org/10.1016/j.envres.2022.113140

Chen, G., Huang, J., Tian, X., Chu, Q., Zhao, Y., Zhao, H., 2018. Effects of influent loads on performance and microbial community dynamics of aerobic granular sludge treating piggery wastewater. J. Chem. Technol. Biotechnol. 93, 1443–1452. https://doi.org/10.1002/jctb.5512

Chen, G.Q., Patel, M.K., 2012. Plastics derived from biological sources: Present and future: A technical and environmental review. Chem. Rev. https://doi.org/10.1021/cr200162d

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2007.01.057

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. https://doi.org/10.1016/j.biotechadv.2007.02.001

Chitapornpan, S., Chiemchaisri, C., Chiemchaisri, W., Honda, R., Yamamoto, K., 2013. Organic carbon recovery and photosynthetic bacteria population in an anaerobic membrane photo-bioreactor treating food processing wastewater. Bioresour. Technol. 141, 65–74. https://doi.org/10.1016/j.biortech.2013.02.048

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

Cogdell, R.J., Isaacs, N.W., Howard, T.D., McLuskey, K., Fraser, N.J., Prince, S.M., 1999. How photosynthetic bacteria harvest solar energy. J. Bacteriol. 181, 3869–3879. https://doi.org/10.1128/jb.181.13.3869-3879.1999

Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., De Gelder, L., 2016. The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J. Appl. Phycol. 28, 2367–2377. https://doi.org/10.1007/s10811-015-0775-2

Costa, S.S., Miranda, A.L., de Morais, M.G., Costa, J.A.V., Druzian, J.I., 2019. Microalgae as source of polyhydroxyalkanoates (PHAs) — A review. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2019.03.099

D'Alessandro, D.M., Smit, B., Long, J.R., 2010. Carbon Dioxide Capture: Prospects for New Materials. Angew. Chemie Int. Ed. 49, 6058–6082. https://doi.org/10.1002/anie.201000431

Dalaei, P., Bahreini, G., Nakhla, G., Santoro, D., Batstone, D., Hülsen, T., 2020. Municipal wastewater treatment by purple phototropic bacteria at low infrared irradiances using a photo-anaerobic membrane bioreactor. Water Res. 173, 115535. https://doi.org/10.1016/j.watres.2020.115535

Dalaei, P., Ho, D., Nakhla, G., Santoro, D., 2019. Low temperature nutrient removal from municipal wastewater by purple phototrophic bacteria (PPB). Bioresour. Technol. 288, 121566. https://doi.org/10.1016/j.biortech.2019.121566

Davis, R., Aden, A., Pienkos, P.T., 2011. Techno-economic analysis of autotrophic microalgae for fuel production. Appl. Energy 88, 3524–3531. https://doi.org/10.1016/j.apenergy.2011.04.018

Demirbas, A., 2007. Progress and recent trends in biofuels. Prog. Energy Combust. Sci. https://doi.org/10.1016/j.pecs.2006.06.001

Dhaouefi, Z., Toledo-Cervantes, A., García, D., Bedoui, A., Ghedira, K., Chekir-Ghedira, L., Muñoz, R., 2018. Assessing textile wastewater treatment in an anoxic-aerobic photobioreactor and the potential of the treated water for irrigation. Algal Res. 29, 170–178. https://doi.org/10.1016/j.algal.2017.11.032

Fan, L., Tu, Z., Chan, S.H., 2021. Recent development of hydrogen and fuel cell technologies: A review. Energy Rep. 7, 8421-8446. https://doi.org/10.1016/j.egyr.2021.08.003

Fradinho, J.C., Oehmen, A., Reis, M.A.M., 2019. Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating conditions. Int. J. Biol. Macromol. 126, 1085–1092. https://doi.org/10.1016/j.ijbiomac.2018.12.270

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

García, D., de Godos, I., Domínguez, C., Turiel, S., Bolado, S., Muñoz, R., 2019. A systematic comparison of the potential of microalgae-bacteria and purple phototrophic bacteria consortia for the treatment of piggery wastewater. Bioresour. Technol. 276, 18–27. https://doi.org/10.1016/J.BIORTECH.2018.12.095

Gilbert, M., Conchedda, G., Van Boeckel, T.P., Cinardi, G., Linard, C., Nicolas, G., Thanapongtharm, W., D'Aietti, L., Wint, W., Newman, S.H., Robinson, T.P., 2015. Income Disparities and the Global Distribution of Intensively Farmed Chicken and Pigs. PLoS One 10, e0133381. https://doi.org/10.1371/journal.pone.0133381

Gilbert, M., Nicolas, G., Cinardi, G., Van Boeckel, T.P., Vanwambeke, S.O., Wint, G.R.W., Robinson, T.P., 2018. Global distribution data for cattle, buffaloes, horses, sheep, goats, pigs, chickens and ducks in 2010. Sci. Data 5, 1–11. https://doi.org/10.1038/sdata.2018.227

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

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

Grassino, M., Batstone, D.J., Yong, K.W.L., Capson-Tojo, G., Hülsen, T., 2022. Method development for PPB culture screening, pigment analysis with UPLC-UV-HRMS vs. spectrophotometric methods, and spectral decomposition-based analysis. Talanta 246, 123490. https://doi.org/10.1016/j.talanta.2022.123490

Hallenbeck, P.C., 2017. Modern topics in the phototrophic prokaryotes: Environmental and applied aspects, Modern Topics in the Phototrophic Prokaryotes: Environmental and Applied Aspects. Springer International Publishing. https://doi.org/10.1007/978-3-319-46261-5

He, S., Lu, H., Zhang, G., Ren, Z., 2021. Production of coenzyme Q10 by purple non-sulfur bacteria: Current development and future prospect. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2021.127326

Ho, S.H., Huang, S.W., Chen, C.Y., Hasunuma, T., Kondo, A., Chang, J.S., 2013. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. Bioresour. Technol. 135, 191–198. https://doi.org/10.1016/j.biortech.2012.10.015

Hole, D.G., Perkins, A.J., Wilson, J.D., Alexander, I.H., Grice, P. V., Evans, A.D., 2005. Does organic farming benefit biodiversity? Biol. Conserv. 122, 113–130. https://doi.org/10.1016/j.biocon.2004.07.018

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

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

Hülsen, T., Batstone, D.J., Keller, J., 2014. Phototrophic bacteria for nutrient recovery from domestic wastewater. Water Res. 50, 18–26. https://doi.org/10.1016/J.WATRES.2013.10.051

Hülsen, T., Hsieh, K., Batstone, D.J., 2019. Saline wastewater treatment with purple phototrophic bacteria. Water Res. 160, 259–267. https://doi.org/10.1016/J.WATRES.2019.05.060

Hülsen, T., Hsieh, K., Lu, Y., Tait, S., Batstone, D.J., 2018a. Simultaneous treatment and single cell protein production from agri-industrial wastewaters using purple phototrophic bacteria or microalgae – A comparison. Bioresour. Technol. 254, 214–223. https://doi.org/10.1016/J.BIORTECH.2018.01.032

Hülsen, T., Hsieh, K., Tait, S., Barry, E.M., Puyol, D., Batstone, D.J., 2018b. White and infrared light continuous photobioreactors for resource recovery from poultry processing wastewater – A comparison. Water Res. 144, 665–676. https://doi.org/10.1016/j.watres.2018.07.040

Hülsen, T., Stegman, S., Batstone, D.J., Capson-Tojo, G., 2022. Naturally illuminated photobioreactors for resource recovery from piggery and chicken-processing wastewaters utilising purple phototrophic bacteria. Water Res. 214, 118194. https://doi.org/10.1016/j.watres.2022.118194

Hunter, C.N., Daldal, F., Thurnauer, M.C., Beatty, J.T. (Eds.), 2009. The Purple Phototrophic Bacteria, Advances in Photosynthesis and Respiration. Springer Netherlands, Dordrecht. https://doi.org/ 10.1007/978-1-4020-8815-5

Iqbal, K., Saxena, A., Pande, P., Tiwari, A., Chandra Joshi, N., Varma, A., Mishra, A., 2022. Microalgae-bacterial granular consortium: Striding towards sustainable production of biohydrogen coupled with wastewater treatment. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2022.127203

Izu, K., Nakajima, F., Yamamoto, K., Kurisu, F., 2001. Aeration conditions affecting growth of purple nonsulfur bacteria in an organic wastewater treatment process. Syst. Appl. Microbiol. 24, 294–302. https://doi.org/10.1078/0723-2020-00027

Jiménez-Ruiz, S., Laguna, E., Vicente, J., García-Bocanegra, I., Martínez-Guijosa, J., Cano-Terriza, D., Risalde, M.A., Acevedo, P., 2022. Characterization and management of interaction risks between livestock and wild ungulates on outdoor pig farms in Spain. Porc. Heal. Manag. 8, 2. https://doi.org/ 10.1186/s40813-021-00246-7

Joo, H.S., Hirai, M., Shoda, M., 2006. Piggery wastewater treatment using *Alcaligenes faecalis* strain No. 4 with heterotrophic nitrification and aerobic denitrification. Water Res. 40, 3029–3036. https://doi.org/10.1016/j.watres.2006.06.021

Kumar, A., Kaur, H., Devi, P., Mohan, V., 2009. Role of coenzyme Q10 (CoQ10) in cardiac disease, hypertension and Meniere-like syndrome. Pharmacol. Ther. https://doi.org/10.1016/j.pharmthera. 2009.07.003

Larimer, F.W., Chain, P., Hauser, L., Lamerdin, J., Malfatti, S., Do, L., Land, M.L., Pelletier, D.A., Beatty, J.T., Lang, A.S., Tabita, F.R., Gibson, J.L., Hanson, T.E., Bobst, C., Torres, J.L.T. y, Peres, C., Harrison, F.H., Gibson, J., Harwood, C.S., 2004. Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. Nat. Biotechnol. 22, 55–61. https://doi.org/10.1038/nbt923

Lee, S.A., Lee, N., Oh, H.M., Ahn, C.Y., 2021. Stepwise treatment of undiluted raw piggery wastewater, using three microalgal species adapted to high ammonia. Chemosphere 263, 127934. https://doi.org/10.1016/j.chemosphere.2020.127934

Lewis, N.S., Nocera, D.G., 2006. Powering the planet: Chemical challenges in solar energy utilization. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.0603395103

Liu, S., Daigger, G.T., Kang, J., Zhang, G., 2019. Effects of light intensity and photoperiod on pigments production and corresponding key gene expression of *Rhodopseudomonas palustris* in a photobioreactor system. Bioresour. Technol. 294, 122172. https://doi.org/10.1016/j.biortech.2019.122172

Liu, S., Zhang, G., Zhang, J., Li, X., Li, J., 2016. Performance, carotenoids yield and microbial population dynamics in a photobioreactor system treating acidic wastewater: Effect of hydraulic retention time (HRT) and organic loading rate (OLR). Bioresour. Technol. 200, 245–252. https://doi.org/10.1016/j.biortech.2015.10.044

Lorenz, R.T., Cysewski, G.R., 2000. Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotechnol. https://doi.org/10.1016/S0167-7799(00)01433-5

Lu, H., Zhang, G., Dai, X., Schideman, L., Zhang, Y., Li, B., Wang, H., 2013. A novel wastewater treatment and biomass cultivation system combining photosynthetic bacteria and membrane bioreactor technology. Desalination 322, 176–181. https://doi.org/10.1016/j.desal.2013.05.007

Lu, H., Zhang, G., Dong, S., 2011a. Quantitative study of PNSB energy metabolism in degrading pollutants under weak light-micro oxygen condition. Bioresour. Technol. 102, 4968–4973. https://doi.org/10.1016/J.BIORTECH.2011.01.027

Lu, H., Zhang, G., Wan, T., Lu, Y., 2011b. Influences of light and oxygen conditions on photosynthetic bacteria macromolecule degradation: Different metabolic pathways. Bioresour. Technol. 102, 9503–9508. https://doi.org/10.1016/j.biortech.2011.07.114

Lu, H., Zhang, G., Zheng, Z., Meng, F., Du, T., He, S., 2019. Bio-conversion of photosynthetic bacteria from non-toxic wastewater to realize wastewater treatment and bioresource recovery: A review. Bioresour. Technol. 278, 383–399. https://doi.org/10.1016/J.BIORTECH.2019.01.070

Lupatini, A.L., Colla, L.M., Canan, C., Colla, E., 2017. Potential application of microalga Spirulina platensis as a protein source. J. Sci. Food Agric. 97, 724–732. https://doi.org/10.1002/jsfa.7987

Madigan, M.T., Bender, K.S., Buckley, D.H., Sattley, W.M., Stahl, D.A., 2021. Brock Biology of Microorganisms. Pearson.

Manyi-Loh, C.E., Mamphweli, S.N., Meyer, E.L., Okoh, A.I., Makaka, G., Simon, M., 2013. Microbial anaerobic digestion (bio-digesters) as an approach to the decontamination of animal wastes in pollution control and the generation of renewable energy. Int. J. Environ. Res. Public Health. https://doi.org/10.3390/ijerph10094390

Marín, D., Carmona-Martínez, A.A., Blanco, S., Lebrero, R., Muñoz, R., 2021. Innovative operational strategies in photosynthetic biogas upgrading in an outdoors pilot scale algal-bacterial photobioreactor. Chemosphere 264, 128470. https://doi.org/10.1016/j.chemosphere.2020.128470

Marín, D., Posadas, E., Cano, P., Pérez, V., Blanco, S., Lebrero, R., Muñoz, R., 2018. Seasonal variation of biogas upgrading coupled with digestate treatment in an outdoors pilot scale algal-bacterial photobioreactor. Bioresour. Technol. 263, 58–66. https://doi.org/10.1016/j.biortech.2018.04.117

Marín, D., Posadas, E., García, D., Puyol, D., Lebrero, R., Muñoz, R., 2019. Assessing the potential of purple phototrophic bacteria for the simultaneous treatment of piggery wastewater and upgrading of biogas. Bioresour. Technol. 281, 10–17. https://doi.org/10.1016/J.BIORTECH.2019.02.073

Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: A review. Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2009.07.020

Medlin, L.K., 2016. Evolution of the diatoms: Major steps in their evolution and a review of the supporting molecular and morphological evidence. Phycologia 55, 79–103. https://doi.org/10.2216/15-105.1

Méndez, L., Sepúlveda-Muñoz, C.A., del Rosario Rodero, M., de Godos, I., Muñoz, R., 2022. Decarbonization potentials using photobiological systems, in: Pathways to Water Sector Decarbonization, Carbon Capture and Utilization. IWA Publishing, pp. 143–170. https://doi.org/10.2166/9781789061796_0143

Mohammadi, M., Soltanzadeh, M., Ebrahimi, A.R., Hamishehkar, H., 2022. *Spirulina platensis* protein hydrolysates: Techno-functional, nutritional and antioxidant properties. Algal Res. 65, 102739. https://doi.org/10.1016/j.algal.2022.102739

Montefiore, L.R., Nelson, N.G., Dean, A., Sharara, M., 2022. Reconstructing the historical expansion of industrial swine production from Landsat imagery. Sci. Rep. 12, 1–12. https://doi.org/10.1038/s41598-022-05789-5

Montes, N., Otero, M., Coimbra, R.N., Méndez, R., Martín-Villacorta, J., 2015. Removal of tetracyclines from swine manure at full-scale activated sludge treatment plants. Environ. Technol. (United Kingdom) 36, 1966–1973. https://doi.org/10.1080/09593330.2015.1018338

Moss, R.H., Edmonds, J.A., Hibbard, K.A., Manning, M.R., Rose, S.K., Van Vuuren, D.P., Carter, T.R., Emori, S., Kainuma, M., Kram, T., Meehl, G.A., Mitchell, J.F.B., Nakicenovic, N., Riahi, K., Smith, S.J., Stouffer, R.J., Thomson, A.M., Weyant, J.P., Wilbanks, T.J., 2010. The next generation of scenarios for climate change research and assessment. Nature. https://doi.org/10.1038/nature08823

Muñoz, R., Guieysse, B., 2006. Algal–bacterial processes for the treatment of hazardous contaminants: A review. Water Res. 40, 2799–2815. https://doi.org/10.1016/J.WATRES.2006.06.011

Muñoz, R., Meier, L., Diaz, I., Jeison, D., 2015. A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading. Rev. Environ. Sci. Biotechnol. https://doi.org/10.1007/s11157-015-9379-1

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

Nagarajan, D., Lee, D.-J., Varjani, S., Lam, S.S., Allakhverdiev, S.I., Chang, J.-S., 2022. Microalgae-based wastewater treatment – Microalgae-bacteria consortia, multi-omics approaches and algal stress response. Sci. Total Environ. 845, 157110. https://doi.org/10.1016/j.scitotenv.2022.157110

Navid, A., Jiao, Y., Wong, S.E., Pett-Ridge, J., 2019. System-level analysis of metabolic trade-offs during anaerobic photoheterotrophic growth in *Rhodopseudomonas palustris*. BMC Bioinformatics 20, 233. https://doi.org/10.1186/s12859-019-2844-z

Obaja, D., MacÉ, S., Mata-Alvarez, J., 2005. Biological nutrient removal by a sequencing batch reactor

(SBR) using an internal organic carbon source in digested piggery wastewater. Bioresour. Technol. 96, 7–14. https://doi.org/10.1016/j.biortech.2004.03.002

Olah, G.A., Prakash, G.K.S., Goeppert, A., 2011. Anthropogenic chemical carbon cycle for a sustainable future. J. Am. Chem. Soc. 133, 12881–12898. https://doi.org/10.1021/ja202642y

Oswald, W.J., 1988. Micro-algae and waste-water treatment. In: Borowitzka, M.B.L. (Ed.), Micro-algal Biotechnology. Cambridge University Press, Cambridge.

Palominos, N., Castillo, A., Guerrero, L., Borja, R., Huiliñir, C., 2021. Coupling of Anaerobic Digestion and Struvite Precipitation in the Same Reactor: Effect of Zeolite and Bischofite as Mg2+ Source. Front. Environ. Sci. 9, 581. https://doi.org/10.3389/fenvs.2021.706730

Perez-Garcia, O., Escalante, F.M.E., De-Bashan, L.E., Bashan, Y., 2011. Heterotrophic cultures of microalgae: Metabolism and potential products. Water Res. 45, 11–36. https://doi.org/10.1016/j.watres.2010.08.037

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

Puyol, D., Hülsen, T., Padrino, B., Batstone, D.J., Martinez, F., Melero, J.A., 2020. Exploring the inhibition boundaries of mixed cultures of purple phototrophic bacteria for wastewater treatment in anaerobic conditions. Water Res. 183, 116057. https://doi.org/10.1016/j.watres.2020.116057

Sakarika, M., Spanoghe, J., Sui, Y., Wambacq, E., Grunert, O., Haesaert, G., Spiller, M., Vlaeminck, S.E., 2019. Purple non-sulphur bacteria and plant production: benefits for fertilization, stress resistance and the environment. Microb. Biotechnol. 1751-7915.13474. https://doi.org/10.1111/1751-7915.13474

Siefert, E., Irgens, R.L., Pfennig, N., 1978. Phototrophic purple and green bacteria in a sewage treatment plant. Appl. Environ. Microbiol. 35, 38–44.

Silveira, C.F., Assis, L.R. de, Oliveira, A.P. de S., Calijuri, M.L., 2021. Valorization of swine wastewater in a circular economy approach: Effects of hydraulic retention time on microalgae cultivation. Sci. Total Environ. 789, 147861. https://doi.org/10.1016/j.scitotenv.2021.147861

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101, 87–96. https://doi.org/10.1263/jbb.101.87

Stomp, M., Huisman, J., Stal, L.J., Matthijs, H.C.P., 2007. Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. ISME J. 1, 271–282. https://doi.org/10.1038/ismej.2007.59

Suzuki, K., Waki, M., Yasuda, T., Fukumoto, Y., Kuroda, K., Sakai, T., Suzuki, N., Suzuki, R., Matsuba, K., 2010. Distribution of phosphorus, copper and zinc in activated sludge treatment process of swine wastewater. Bioresour. Technol. 101, 9399–9404. https://doi.org/10.1016/j.biortech.2010.07.014

Szulczyk, K.R., Tan, Y.M., 2022. Economic feasibility and sustainability of commercial bioethanol from microalgal biomass: The case of Malaysia. Energy 253, 124151. https://doi.org/10.1016/j.energy.2022.124151

Torres-Franco, A.F., Zuluaga, M., Hernández-Roldán, D., Leroy-Freitas, D., Sepúlveda-Muñoz, C.A., Blanco, S., Mota, C.R., Muñoz, R., 2021. Assessment of the performance of an anoxic-aerobic microalgal-bacterial system treating digestate. Chemosphere 270, 129437. https://doi.org/10.1016/j.chemosphere.2020.129437

Wang, X., Zhu, M., Wang, K., He, S., Shi, X., Yuan, B., Dong, B., Wang, Z., 2022. Preparation of core-shell microcapsules based on microfluidic technology for the encapsulation, protection and controlled delivery of phycocyanin. J. Drug Deliv. Sci. Technol. 72, 103361. https://doi.org/10.1016/j.jddst.2022.103361

Wen, S., Liu, H., He, H., Luo, L., Li, X., Zeng, G., Zhou, Z., Lou, W., Yang, C., 2016. Treatment of anaerobically digested swine wastewater by *Rhodobacter blasticus* and *Rhodobacter capsulatus*. Bioresour. Technol. 222, 33–38. https://doi.org/10.1016/j.biortech.2016.09.102

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

Yang, A., Zhao, W., Peng, M., Zhang, G., Zhi, R., Meng, F., 2018. A special light-aerobic condition for photosynthetic bacteria-membrane bioreactor technology. Bioresour. Technol. 268, 820–823. https://doi.org/10.1016/j.biortech.2018.08.008

Zabed, H.M., Akter, S., Yun, J., Zhang, G., Zhang, Y., Qi, X., 2020. Biogas from microalgae: Technologies, challenges and opportunities. Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2019.109503

Zhu, Y., Lu, W., Ye, L., Chen, Z., Hu, W., Wang, C., Chen, J., Yu, H., 2017. Enhanced synthesis of Coenzyme Q10 by reducing the competitive production of carotenoids in *Rhodobacter sphaeroides*. Biochem. Eng. J. 125, 50–55. https://doi.org/10.1016/j.bej.2017.03.019

Aims and scope of the thesis

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2.1. Justification of the thesis

The rapid increase in human population has also led to an increasing demand for food worldwide. In this context, intensive livestock farming has also grown in recent years in order to satisfy this increasing demand for food and animal protein, which has resulted in an increase in wastewater production and environmental contamination. In particular, water bodies such as rivers and lakes are nowadays severely affected by the increasing contamination caused by the uncontrolled discharge of livestock wastewaters. Thus, new biotechnologies should be engineered to efficiently treat and valorize livestock wastewaters in order to achieve a sustainable development at global scale. Livestock wastewaters such as PWW are typically characterized by high concentrations of organic matter and ammonium, which have been traditionally treated in anaerobic lagoon, activated sludge processes or closed anaerobic digesters. However, these technologies are not capable of sustainably treating PWW. In this regard, the use of photosynthetic microorganisms has received an increasing attention in the past decade due to their extraordinary metabolic capabilities for nutrient and carbon recovery from wastewaters. Purple phototrophic bacteria and microalgae are able to obtain energy by the absorption of solar photons during the photosynthesis process. In addition, PPB can grow and obtain energy via oxidative phosphorylation (by chemotrophic growth) and are able to grow on organic molecules or by fixing molecules from the atmosphere, via heterotrophic and autotrophic growth, respectively. On the other hand, microalgae perform oxygenic photosynthesis and can fix carbon dioxide and organic pollutants under autotrophic and heterotrophic growth, respectively. These photosynthetic microorganisms can support consistent carbon, nitrogen and phosphorous removals from PWW. However, the influence of key environmental and operational parameters on PPB-based PWW treatment under batch and continuous mode remained unexplored. In brief, this thesis systematically assessed and optimize the potential of PPB assisted with microalgae for PWW treatment as a low-cost and environmentally sustainable solution for a sustainable management of livestock wastewaters.

2.2. Main objectives

The global objective of the present thesis was the assessment and optimization of the performance of purple phototrophic bacteria for PWW treatment. This work focused on the systematic evaluation of the influence of environmental, operational and design parameters on PWW treatment under batch and continuous mode in photobioreactors. This global aim was addressed through the following specific objectives:

Objective 1. Systematic evaluation of the performance of piggery wastewater treatment using PPB under different PWW loads, anaerobic and aerobic conditions and pH control in batch photobioreactors.

Objective 2. Systematic evaluation of the performance of PPB under different types of radiation, temperature and type of inoculum during PWW treatment in batch photobioreactors.

Objective 3. Comparative evaluation and optimization of the long-term performance of continuous piggery wastewater treatment in open and closed PPB-based photobioreactors.

Objective 4. Evaluation and optimization of carbon and nitrogen removal from piggery wastewater in sequential PPB and microalgal-bacterial photobioreactors.

2.3. Development of the thesis

In the present thesis, an assessment and optimization of piggery wastewater treatment using PPB was conducted. In the **introduction** section, a detailed and updated literature review of the state of the art of wastewater treatment using purple phototrophic bacteria and microalgae was presented. In **aims and objectives** section, the justification of this thesis work, along with its general and specific objectives, are reviewed. **Chapter 1 to 4** are devoted to present and discuss the experimental investigations conducted in this PhD thesis. Thus, the evaluation of the performance of PWW treatment using PPB was carried out in batch photobioreactors (**chapter 1 and 2**) and in continuous photobioreactors (**chapter 3 and 4**). The assessment of the influence of PWW loads, oxygen and inorganic carbon supplementation, and pH on PPB-based PWW was conducted in batch photobioreactors using near-infrared radiation (NIR) as energy source in order to fulfill objective 1 (**chapter 1**). Additional batch tests were performed to assess the influence of the type of radiation (photosynthetically active radiation (PAR) vs near-infrared radiation (NIR) vs PAR filtered using a UV-VIS filter), temperature (13 °C and 30°), type of metabolism (photoheterotrophic and chemoheterotrophic) and type of inoculum (mixed cultures and strain *Rhodopseudomonas palustris*) on PPB-based PWW treatment by PPB in order to fulfill objective 2 (**chapter 2**). On the other hand, the long-term performance of PPB during the continuous PWW treatment in photobioreactor was evaluated. Thus, the influence of PWW dilutions (1:4 and 1:8), ratio of illuminated area to volume of the photobioreactor, biomass settling and recirculation were evaluated in PPB open and closed photobioreactors during the continuous PWW treatment to achieve objective 3 (**chapter 3**). The performance of an innovative configuration composed of an anaerobic PPB photobioreactor coupled to a sequential aerobic microalgae-bacteria photobioreactor operated at different hydraulic retention times (12.2 and 6.2 days) and intensities of near-infrared radiation in the PPB photobioreactor (30 and 114 W m^{-2}) was assessed during the PWW treatment under continuous operation to fulfill objective 4 (**chapter 4**). Finally, the **conclusions and future work** section summarizes the main contributions of this thesis and the future research based on the presented outcomes.

Chapter 1

A systematic optimization of piggery wastewater treatment with purple phototrophic bacteria.

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Chapter 2

Exploring the metabolic capabilities of purple phototrophic bacteria during piggery wastewater treatment.

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Chapter 3

Comparative evaluation of continuous piggery wastewater treatment in open and closed purple phototrophic bacteria-based photobioreactors.

Cristian A. Sepúlveda-Muñoz, Roxana Ángeles, Ignacio de Godos, Raúl Muñoz. (2020). J Water Process Eng. 38: 101608.

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Chapter 4

Photosynthetic treatment of piggery wastewater in sequential purple phototrophic bacteria and microalgae-bacteria photobioreactors.

Cristian A. Sepúlveda-Muñoz, Gorka Hontiyuelo, Saúl Blanco, Andrés F. Torres-Franco, Raúl Muñoz. (2022). J Water Process Eng. 47: 102825.

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Photosynthetic treatment of piggery wastewater in sequential purple phototrophic bacteria and microalgae-bacteria photobioreactors.

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Conclusions and future work

Conclusions and future work

A systematic evaluation of the influence of both environmental conditions, photobioreactor configurations and operational parameters on the treatment of piggery wastewater using purple phototrophic bacteria and microalgae was conducted. The results obtained confirmed the promising potential of photosynthetic microorganisms for resource recovery during piggery wastewater treatment.

A preliminary evaluation of the potential of PPB for piggery wastewater treatment and the optimization of environmental and operational parameters was conducted in batch photobioreactors, as described in **chapter 1 and 2**. These key parameters were implemented to optimize the photobioreactor configuration during the continuous treatment of PWW using PPB and microalgae, as described in **chapter 3 and 4**.

C**hapter 1** showed that PWW dilution was required to prevent the inhibition of PPB as a result of the toxic effects of the high ammonium concentrations present in PWW and to favour a more effective penetration of the radiation into the culture broth, thus enhancing photosynthetic capacity. PPB were able to grow under both anaerobic and aerobic conditions. However, nutrient recovery was favoured under anaerobic conditions, where assimilation in form of PPB biomass was identified as the main mechanism of nutrient assimilation from PWW. CO_2 addition was also identified as an effective operational strategy to maximize carbon removal from PWW along with PPB growth. However, the beneficial effects from CO_2 supplementation derived from the neutral pH control, which is the actual key control parameter on PPB-based PWW valorization.

C**hapter 2** demonstrated that PPB were able to effectively grow under photosynthetically active radiation (PAR), PAR filtered with UV-VIS absorbing foil and near-infrared radiation (NIR) without significant differences under photoheterotrophic metabolism. The use of PAR filtered with UV-VIS absorbing foil represented the most cost-effective source of NIR specific for PPB growth. In addition, PPB were able to grow at 13 °C and support similar removal efficiencies than at 30 °C. PPB were not able to grow under chemoheterotrophic metabolism under anaerobic conditions during PWW treatment in the absence of NIR supply. Moreover, mixed PPB cultures were more efficient than the pure strain *Rhodopseudomonas palustris* in terms of nutrient removal efficiency, which confirmed the promising metabolic capabilities of mixed PPB cultures during PWW treatment.

C**hapter 3** confirmed the long-term efficiency of PPB-based piggery wastewater treatment. The open photobioreactor supported higher nutrient removals than the closed photobioreactor, which was mediated by the larger contribution of abiotic mechanisms such as CO_2 and NH_3 stripping. In addition, the open photobioreactor exhibited higher water evaporation losses compared to the closed system. The decrease in PWW load did not entail an enhancement in process performance in both photobioreactors, while the increase in the illuminated area to volume ratio induced higher nutrient removals. In addition, biomass settling and recirculation resulted in enhanced nitrogen removals, while the increase in infrared radiation favoured PPB growth by enhancing the photosynthetic process. Finally, enclosed photobioreactor configuration, PWW dilution and process operation with high illuminated areas supported beneficial effects on PPB growth and PWW treatment.

C**hapter 4** showed the potential of a novel photobioreactor configuration coupling a PPB-PBR to a MB-PBR for simultaneous carbon and nitrogen removal. The efficient photoheterotrophic metabolism of PPB supported a high carbon assimilation, while microalgal-bacterial activity enhanced nitrogen and total solid suspended removal during continuous PWW treatment. High hydraulic retention times prevented biomass washout, whereas an increase in the near-infrared radiation during operation at lower HRTs enhanced photosynthetic activity in PPB, thus contributing to an overall enhancement of the PWW mediated treatment. The PPB photobioreactor was dominated mainly by *Rhodopseudomonas* sp., while the sequential microalgae-bacteria photobioreactor configuration favoured the dominance of the microalga *Mychonastes homosphaera*. In combination, both photosynthetic microorganisms supported a high assimilation of carbon and nitrogen in the form of biomass as the primary removal mechanism. Overall, PPB-PBR emerged as an effective pretreatment for microalgae-based treatment of PWW, thus demonstrating the high potential of combing PPB and microalgae during PWW treatment.

Despite the promising advances obtained in this thesis based on piggery wastewater treatment using purple phototrophic bacteria, the future research needed to move this technology to commercial scale should focus on:

- Optimization of PWW treatment in PPB photobioreactors under **outdoor conditions in a relevant environment (TRL6)**, which will allow evaluating the effects of direct solar irradiation, daily and seasonal changes in light intensities and temperature, changes in PWW composition and microbial contamination.

- S**cale-up** of continuous photobioreactors treating PWW using purple phototrophic bacteria in relevant environments.

- Assessing the potential of the PPB biomass generated from PWW treatment under outdoors conditions in a relevant environment for the **production of protein, biopolymers, biofuels, biofertilizers and high added value products**.

- **Techno-economic evaluation** of PPB-based PWW treatment coupled with biomass valorization to assess the potential contribution of this platform technology to the creation of a circular economy in the field of animal farming.

About the author

Biography

Cristian A. Sepúlveda-Muñoz (Santiago, Chile, 1990) studied Biotechnology at the University of Antofagasta (Antofagasta, Chile). His BSc thesis, which was focused on the use of *Chlamydomonas reinhardtii* as a feedstock for biogas production by anaerobic digestion, was conducted under the supervision of Dra. Mariella Rivas and Dr. Tomáš Podzimek in laboratory of Algal Biotechnology and Sustainability at the University of Antofagasta in collaboration with the Scientific and Technological Bioresource Nucleus (BioRen) at the University of la Frontera (Temuco, Chile) under the supervision of Dr. David Jeison. Cristian studied a MSc in Biotechnology at the University of Antofagasta with a thesis entitled "Biophotovoltaics: pigments of microalgae isolated from the Atacama Desert as photosensitizers in dye-sensitized solar cell", which was conducted under the supervision of Dra. Mariella Rivas at the laboratory of Algal Biotechnology & Sustainability at the University of Antofagasta in collaboration with the BioNanotechnology and Microbiology Lab at the University Andrés Bello (Santiago, Chile) under the supervision of Dr. José Manuel Pérez-Donoso. In 2018, Cristian was awarded a fellowship under Formation of Advanced Human Capital Program of CONICYT (Chile) and joined the Environmental Technology Research Group and the Institute of Sustainable Processes at the University of Valladolid to conduct his PhD studies in Chemical and Environmental Engineering. His PhD thesis, focused on piggery wastewater treatment using purple phototrophic bacteria, was supervised by Dr. Raúl Muñoz and Dr. Ignacio de Godos.

Publications in ISI-indexed journals

Within the scope of this PhD thesis:

Cristian A. Sepúlveda-Muñoz, Ignacio de Godos, Daniel Puyol, Raúl Muñoz. (2020). A systematic optimization of piggery wastewater treatment with purple phototrophic bacteria. *Chemosphere*. 253: 126621.

Cristian A. Sepúlveda-Muñoz, Roxana Ángeles, Ignacio de Godos, Raúl Muñoz. (2020). Comparative evaluation of continuous piggery wastewater treatment in open and closed purple phototrophic bacteria-based photobioreactors. *J Water Process Eng*. 38: 101608.

Cristian A. Sepúlveda-Muñoz, Gorka Hontiyuelo, Saúl Blanco, Andrés F. Torres-Franco, Raúl Muñoz. (2022). Photosynthetic treatment of piggery wastewater in sequential purple phototrophic bacteria and microalgae-bacteria photobioreactors. *J Water Process Eng*. 47: 102825.

Cristian A. Sepúlveda-Muñoz, Andrés F. Torres-Franco, Ignacio de Godos, Raúl Muñoz. (2022). Exploring the metabolic capacities of purple phototrophic bacteria during piggery wastewater treatment. (Submitted for publication in *J Water Process Eng*).

Cristian A. Sepúlveda-Muñoz, José Gonçalves, Elisa Rodríguez, Raúl Muñoz. (2023). Comparative evaluation of bacterial community dynamics in open and closed purple phototrophic bacteria-based photobioreactors treating piggery wastewater. (In preparation).

Out of the scope this PhD thesis:

Roxana Ángeles, Esther Arnaiz, Julia Gutiérrez, **Cristian A. Sepúlveda-Muñoz**, Oscar Fernández-Ramos, Raúl Muñoz, Raquel Lebrero. (2020). Optimization of photosynthetic biogas upgrading in closed photobioreactors combined with algal biomass production. *J Water Process Eng*. 38: 101554.

Andrés F. Torres-Franco, Maribel Zuluaga, Diana Hernández-Roldán, Deborah Leroy-Freitas, **Cristian A. Sepúlveda-Muñoz**, Saúl Blanco, César R. Mota, Raúl Muñoz. (2021). Assessment of the performance of an anoxic-aerobic microalgal-bacterial system treating digestate. *Chemosphere*. 270: 129437.

Martin Struk, **Cristian A. Sepúlveda-Muñoz**, Ivan Kushkevych, Raúl Muñoz. (2022). Photoautotrophic removal of hydrogen sulfide from biogas using purple and green sulfur bacteria. (Submitted for publication in *J Hazard Mater*).

Book Chapters:

Lara Méndez, **Cristian A. Sepúlveda-Muñoz**, María del Rosario Rodero, Ignacio de Godos, Raúl Muñoz. (2022). Decarbonization potentials using photobiological systems. In: Ren, Z. J. and Pagilla, K. (Ed.), Pathways to Water Sector Decarbonization, Carbon Capture and Utilization. IWA Publishing, London, UK. ISBN: 9781789061796.

Poster communications:

<u>**Cristian A. Sepúlveda-Muñoz**</u>, Gorka Hontiyuelo, Saúl Blanco, Andrés F. Torres-Franco, Raúl Muñoz. Photosynthetic treatment of piggery wastewater in sequential purple phototrophic bacteria and microalgae photobioreactors. XIV Reunión Jóvenes Investigadores Iberoamericanos. 30 March 2022, Tordesillas (Spain).

Research internships supervision

John Precious Nnam, student of the Master in Environmental Engineering at University of Valladolid. Project title: Wastewater treatment using purple photosynthetic bacteria. Academic year 2019/20.

Gorka Hontiyuelo Lomas, student of the Bachelor Degree in Chemical Engineering at University of Valladolid. Project title: Wastewater treatment using purple phototrophic bacteria and microalgae. Academic year 2020/21.

Martin Struk, student of the PhD programme in Microbiology from Masaryk University. Project title: Optimization of conditions for cultivation of photolithotrophic sulfur bacteria and their use for biogas purification. Academic year 2020/21.

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