


# Wastewater Treatment Using Photosynthetic Microorganisms

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**Abstract:** Wastewaters are mainly classified as domestic, industrial and agro-industrial based on their production source. Piggery wastewater (PWW) is a livestock wastewater characterized by its high concentrations of organic matter and ammonium, and by its odour nuisance. Traditionally, PWW has been treated in open anaerobic lagoons, anaerobic digesters and activated sludge systems, which exhibit high greenhouse gas emissions, a limited nutrients removal and a high energy consumption, respectively. Photosynthetic microorganisms can support a sustainable wastewater treatment in engineered photobioreactors at low operating costs and with an efficient recovery of carbon, nitrogen and phosphorous. These microorganisms are capable of absorbing solar irradiation through the photosynthesis process to obtain energy, which is used for their growth and associated carbon and nutrients assimilation. Purple phototrophic bacteria (PPB) represent the photosynthetic microorganisms with the most versatile metabolism in nature, whereas microalgae are the most-studied photosynthetic microorganisms in recent years. This review describes the fundamentals, symmetry and asymmetry of wastewater treatment using photosynthetic microorganisms such as PPB and microalgae. The main photobioreactor configurations along with the potential of PPB and microalgae biomass valorisation strategies are also discussed.

**Keywords:** biomass valorisation; microalgae; nutrient recovery; purple phototrophic bacteria; swine manure



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## 1. Introduction

The human population in the world reached 8 billion in 2022 and recent projections suggested a population up to 9.7 billion by 2050, which represents future challenges for the sustainable development of mankind [1]. This demographic expansion will entail global changes generated by an intensive anthropogenic activity on the Earth. 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 with an extensive innovation in wastewater treatment technology [2,3].

Wastewaters are mainly classified depending on their origin as domestic, industrial and agro-industrial [4]. Depending on their origin, wastewaters exhibit a different physical, chemical and microbiological composition [5]. Domestic wastewaters are generated from human activities and characterized by low concentrations of organic matter and nutrients (Table 1) [6]. 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 high concentrations of carbon (sugars and alcohols) [7], whereas wastewater generated by the mining industry contains highly toxic and recalcitrant

pollutants such as heavy metals. 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 identified due to the elevated content of carbon from animal manure and a high content of ammonium from animal urine (Table 1) [8,9].

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

Composition	Domestic	Industrial <sup>1</sup>	Agro-Industrial <sup>2</sup>
COD (mg L <sup>-1</sup> )	526	10,000	54,000
TN (mg L <sup>-1</sup> )	46	1	5000
TP (mg L <sup>-1</sup> )	6	3	1500
pH	6.8	7.0	7.7

<sup>1</sup> Industrial wastewater represented by brewery wastewater [7]. <sup>2</sup> Agro-industrial wastewater represented by piggery wastewater [8].

## 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. Pigs production in the EU amounted 23,720 thousand metric tons of pig meat in 2021, with Spain as the second largest pork producer in the EU [10]. Other countries such as China and United States produced 47,500 and 12,559 thousand metric tons of pig meat in 2021, respectively (United States, Department of Agriculture). Intensive animal husbandry generates large volumes of wastewaters with a significant content of carbon, 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 [3,8,11,12]. Piggery wastewater (PWW) is considered one of the most polluting agro-industrial wastewaters [8], characterized by high concentrations of organic matter, volatile fatty acids (carbon source), NH<sub>4</sub><sup>+</sup> (nitrogen source) and even presence of heavy metals (such as copper, lead and zinc) [8,13,14]. In addition, the inadequate management of this wastewater can cause malodour 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 cage washing and maintenance (Table 2). Overall, these wastewaters are identified due to elevated concentration of dissolved carbon, nitrogen and phosphorus, and suspended solids.

On the other hand, drugs (such as antiparasitics and antibiotics) are intensively applied during pigs farming to maintain animal health. This represents a serious problem because it is estimated that between 30–90% of the supplemented drugs are excreted without change in their chemical structure, promoting high concentrations of drugs in PWW [15]. Traditionally, these compounds are referred to as emerging contaminants and entail potential risks to the environment and humans [16]. Emerging contaminants such as the antiparasitic fenbendazole at concentrations of 396 ng L<sup>-1</sup>, the antibiotics of the  $\beta$ -lactams family such as penicillin G (up to 0.03 mg L<sup>-1</sup>), tetracyclines such as doxycycline (up to 0.16 mg L<sup>-1</sup>), fluoroquinolones such as enrofloxacin (up to 1.65 mg L<sup>-1</sup>) and sulfonamides such as sulfadimidine (up to 0.06 mg L<sup>-1</sup>) have been detected in PWW [17]. These emerging pollutants can be released to the environment if the PWW is not properly treated, thus triggering the risk of pathogenic bacteria evolution on antibiotics resistance.

**Table 2.** Pollutant concentrations in PWW from different farm around the world.

Farm	COD (mg L <sup>-1</sup> )	TN (mg L <sup>-1</sup> )	TP (mg L <sup>-1</sup> )	TSS (mg L <sup>-1</sup> )	Reference
Daejeon (South Korea)	18,700	810	290	-	[18]
Barcelona (Spain)	7450	785	120	3100	[19]
Yokohama (Japan)	5300	1270	-	-	[20]
Castilla y León (Spain)	54,000	5000	1500	-	[8]
Seosan-si (South Korea)	8420	1150	34	-	[21]
Santiago (Chile)	18,400	1085	172	-	[22]
Queensland (Australia)	4130	1160	160	2420	[3]

### 3. Conventional 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 implemented approaches for PWW management is the use of single-stage or multi-stage anaerobic lagoons to store and treat PWW [23]. This approach is responsible for the release to the open atmosphere of high concentrations of greenhouse gases such as CO<sub>2</sub>, CH<sub>4</sub>, NH<sub>3</sub> and H<sub>2</sub>S, with several environmental adverse effects. The decanted manure from these anaerobic lagoons is typically transformed into a biofertilizer with a high concentration of nitrogen and phosphorous, despite the fact 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 [24].

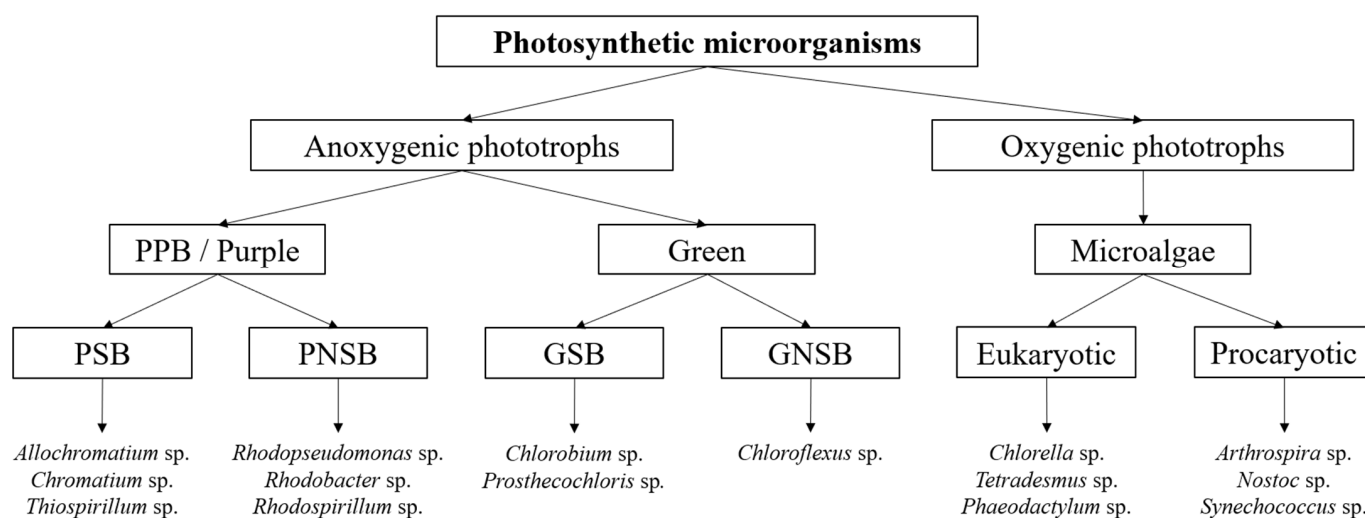
Another conventional technology for PWW treatment is anaerobic digestion (AD). This bioprocess is carried out by bacteria and archaea in the absence of O<sub>2</sub> or oxidized forms of nitrogen. Those microorganisms degrade the organic matter present in PWW producing mainly methane (CH<sub>4</sub>) at concentrations ranging from 40 to 75%, carbon dioxide (CO<sub>2</sub>) at concentrations of 15 to 60% and other gases at lower concentrations such as hydrogen (H<sub>2</sub> > 1%), ammonium (NH<sub>3</sub> > 1%), oxygen (O<sub>2</sub> > 1%) and hydrogen sulphide (H<sub>2</sub>S > 3%) [25–27]. This type of technology allows the conversion of PWW into a renewable gas energy product, namely biogas. However, AD is 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-degradable part of PWW and the anaerobic biomass formed in the process, which must be disposed of in landfills prior to composting.

Activated sludge treatment is an aerobic process that is also typically used for PWW treatment despite its high energy demand [28]. This process consists of an initial removal of suspended solids followed by an aerobic bioreactor with activated sludge, and a final step of settling to collect the biomass generated [29]. The aerobic tank, where carbon is oxidized to CO<sub>2</sub> and H<sub>2</sub>O, and ammonium to nitrite or nitrate, can be preceded by an anoxic tank devoted to heterotrophic denitrification (NO<sub>3</sub> to N<sub>2</sub>). 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 associated high energy demand. In addition, another limitation of activated sludge processes derives

from the fact that carbon and nitrogen removal is carried out via volatilization into the atmosphere. Indeed, the organic matter present in PWW is converted into CO<sub>2</sub>, which contributes to the production of greenhouse gases, whereas nitrogen is converted into N<sub>2</sub> through nitrification and denitrification processes or volatilize as NH<sub>3</sub>. Finally, the heavy metals such as copper and zinc present in PWW are retained in the biomass generated, which is discarded in landfills.

#### 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 [3,8,12,30–33]. 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) (Figure 1). This group is subdivided into two bacterial group: purple sulphur bacteria (PSB) and purple non-sulphur bacteria (PNSB) [34]. PPB were originally classified based on their sulphide 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* [35]. Another photosynthetic bacterial group capable of performing anoxygenic photosynthesis are green sulphur bacteria (GSB) and green non-sulphur bacteria (GNSB), classified in these groups according to their sulphur tolerance and their characteristic green colour [27].



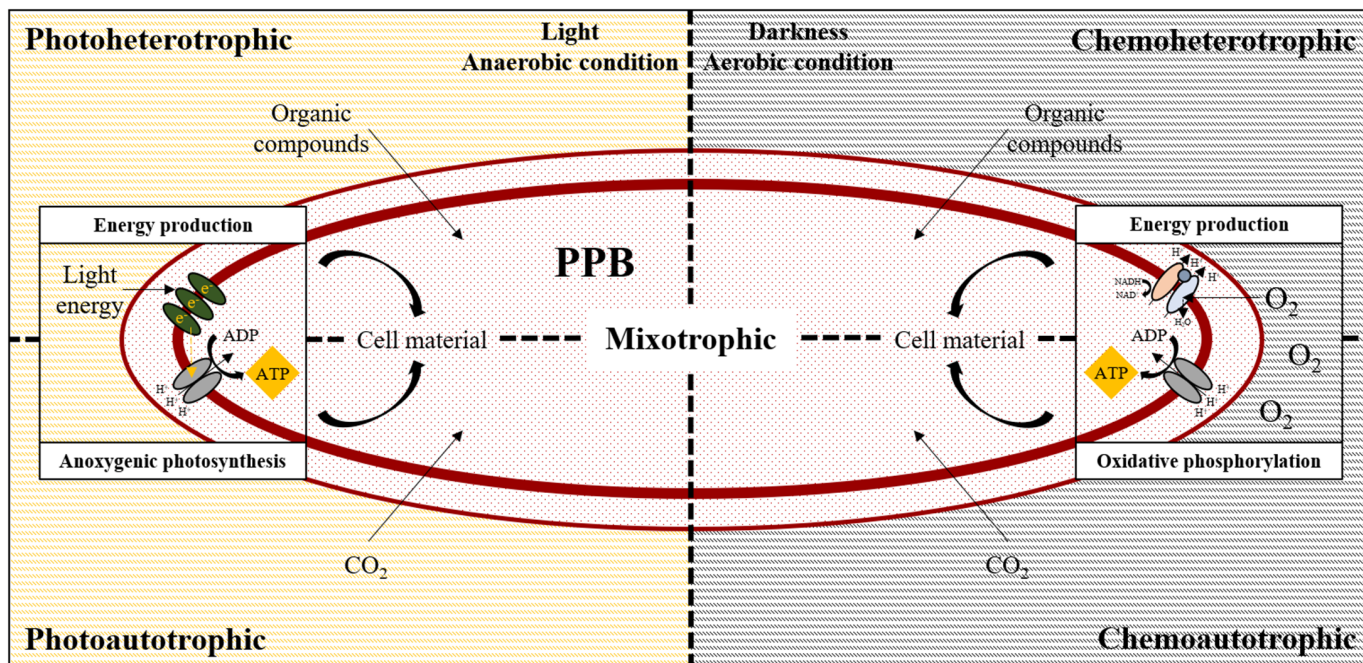
**Figure 1.** Diagram of photosynthetic microorganisms classification performing anoxygenic or oxygenic photosynthesis.

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) [36]. 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* [37]. 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 [9,35,38,39].

#### 4.1. PPB-Based Treatment

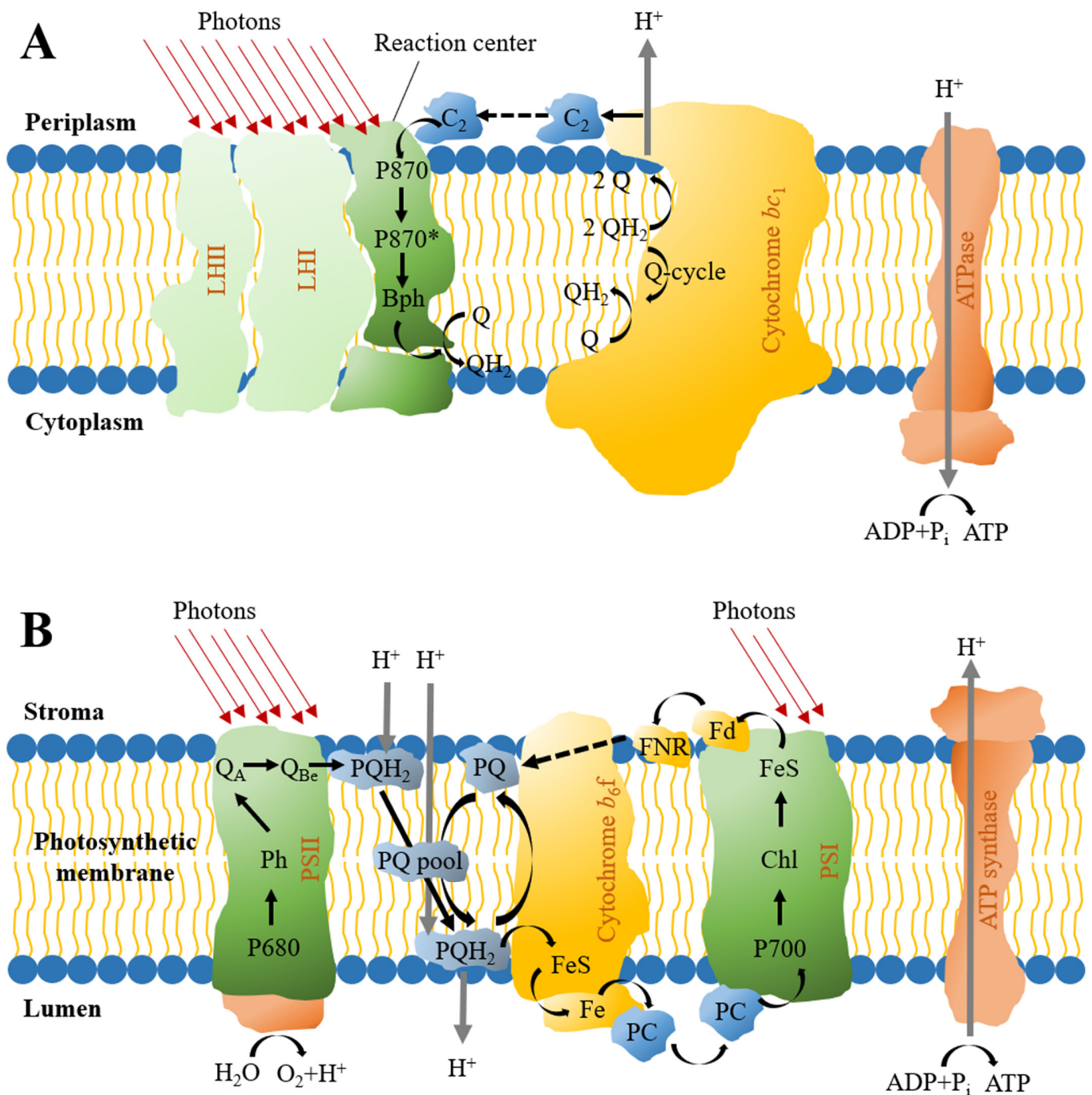
PPB are gram-negative bacteria, prokaryotes and have the most versatile metabolism among microorganisms (Figure 2). *Rhodospirillum rubrum*, *Rhodospirillum rubrum* from the PNSB group, and *Allochroamatium vinosum* and *Thiocapsa roseopersicina* corresponding from the PSB group ranked among the most investigated PPB [35,40]. PNSB are capable of growing under anaerobic and aerobic conditions via phototrophic and chemotrophic metabolisms, respectively [14,41,42]. 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<sub>2</sub> or S<sub>2</sub>O<sub>3</sub><sup>-2</sup> as electron donors [35]. PPB can grow in environments with extreme conditions: high concentrations of salt [43], extremely high temperatures or low temperatures in the range of 10–13 °C [9,44,45] and very acid or alkaline conditions [38].



**Figure 2.** Metabolic diagram of purple phototrophic bacteria, based on the genome of *Rhodospirillum rubrum* (adapted from [46]).

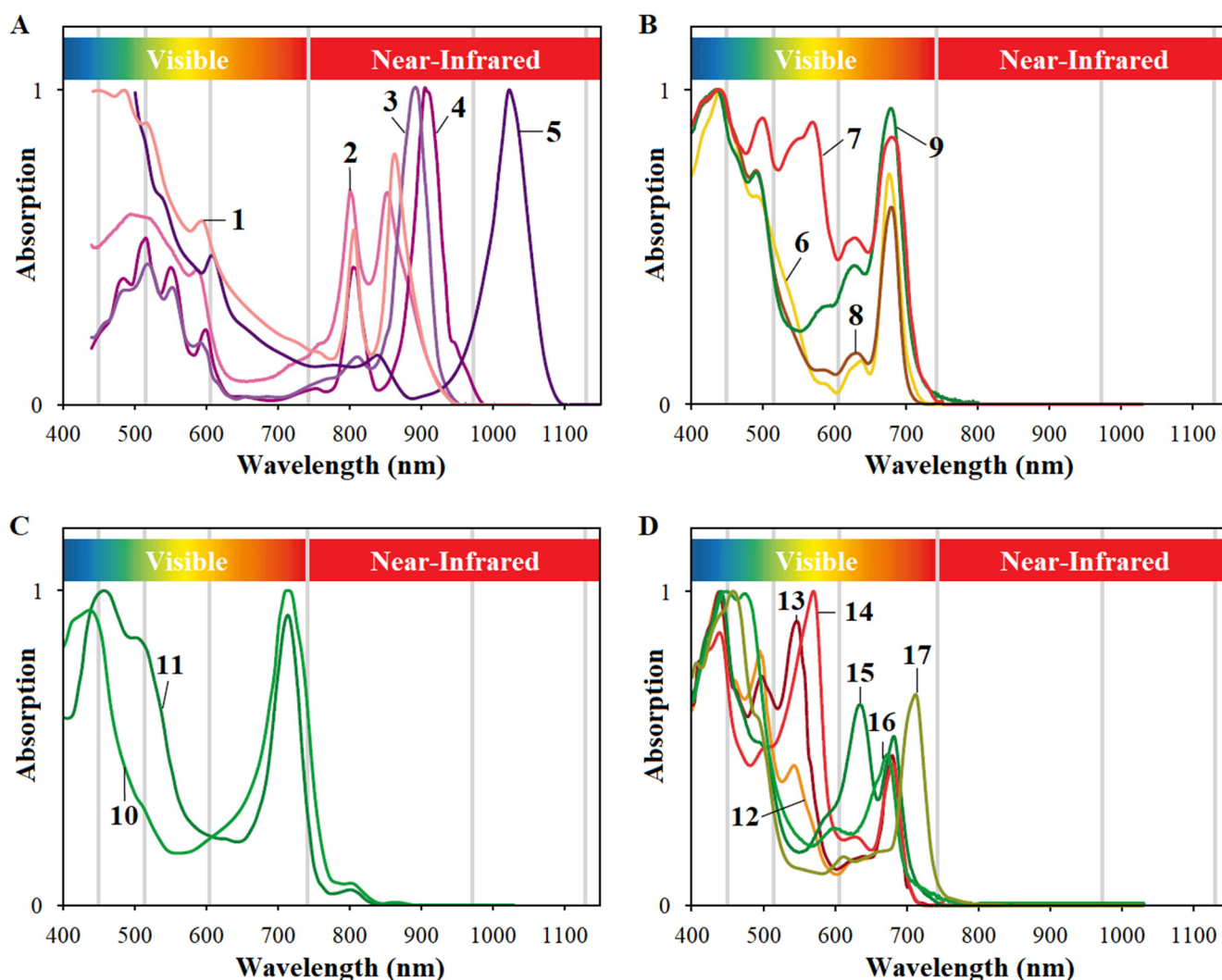
PPB can obtain energy through the anoxygenic photosynthesis process, absorbing energy from the sunlight radiation reaching the earth surface by photon absorption using photosynthetic complexes. In this process, PPB produce ATP via photophosphorylation under phototrophic growth [34,35]. Indeed, PPB are more efficient photosynthetic microorganisms in the photoconversion compared to microalgae [6]. The first descriptions of the basic mechanisms underlying photosynthesis were based on investigation using PPB as model microorganisms [47]. Electron flow during the photosynthetic process in PPB begins with photon 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\*, Figure 3), it becomes a very strong electron donor, capable of transferring energized 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 synthesizes 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  $C_2$ , and return to the reaction centre P870, closing the cyclic photophosphorylation in PPB [39].



**Figure 3.** 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 [39]).

PPB are a unique group of microorganisms capable of absorbing radiation of wavelengths in the near-infrared (NIR) spectrum (Figure 4). 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 [48]. 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 [9].



**Figure 4.** Absorption spectrum (400–1150 nm) of the purple phototrophic bacteria (A) *Rhodobacter capsulatus* (1), *Rhodobacter sphaeroides* (2), *Rhodospirillum rubrum* (3), *Roseospirillum parvum* (4), *Blasotchloris 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 phaeoathratiforme* (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 [48]).

On the other hand, PPB can grow aerobically under chemoheterotrophic or chemoautotrophic metabolisms, obtaining energy via oxidative phosphorylation (Figure 2) by degrading macromolecules for ATP synthesis [49,50]. Molecular oxygen is used as an electron acceptor under these growth conditions. However, this O<sub>2</sub> causes a repression and inhibition of bacteriochlorophylls synthesis, which harms the photosynthetic capacity of PPB [51].

*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<sub>2</sub> via the Calvin–Benson–Bassham (CBB) cycle [41], which confers this microorganism an efficient metabolism for the assimilation of alcohols, amino acids, carbohydrates, fatty acids, organic acid and even recalcitrant pollutants in wastewater [9,34]. 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<sub>2</sub> produced from acetate oxidation is fixed and used for biomass production [52]. In addition, PPB can assimilate all forms inorganic of nitrogen such as NO<sub>3</sub><sup>−</sup>, NO<sub>2</sub><sup>−</sup>, N<sub>2</sub> and NH<sub>4</sub><sup>+</sup> [53]. This ability to fix N<sub>2</sub> 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<sub>2</sub> and generates NH<sub>3</sub> 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 such as cyclohexane carboxylate, toluene, benzoate and their derivatives [35]. On the other hand, due to their extraordinary metabolism PPB are able to remove emerging contaminants such as penicillin G up to 68%, doxycycline up to 38% and enrofloxacin removal up to 88% [17]. 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 [34,53]. For instance, concomitant removal efficiencies of 63% for COD, 99% for NH<sub>4</sub>-N and 88% PO<sub>4</sub>-P have been recorded during domestic wastewater treatment using PPB in batch tests with acetate supplementation [6]. In addition, domestic wastewater treatment by PPB under low radiation intensities (<3 W m<sup>−2</sup>) with high removal efficiencies over 90% for COD and up to 86% and 91% for TN and TP removal, respectively, has been reported in the literature [54]. 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% [45]. Similarly, high COD removal efficiencies have been reported during industrial wastewater treatments with PPBs: COD removal of 96% in brewery wastewater [55], 90% in acidic food wastewater [56] and 89% in VFA-rich food industry wastewater [57]. On the other hand, PPB can also support satisfactory organic matter and nutrient removals during agro-industrial wastewaters treatment. For instance, PPB provided removal efficiencies higher than 90% for COD and TN during poultry processing wastewater treatment [32] and higher than 83% for COD during digested piggery wastewater treatment [58]. In addition, PPB exhibit a remarkable metabolism to remove carbon from PWW, with removal efficiencies ranging from 71% to 99% (Table 3). This is mainly due to the fact that the main carbon source present in PWW are volatile fatty acids (VFAs), where CH<sub>3</sub>COOH, CH<sub>3</sub>-CH<sub>2</sub>-CH<sub>2</sub>-COOH, CH<sub>3</sub>-CH<sub>2</sub>-COOH and (CH<sub>3</sub>)<sub>2</sub>CHCO<sub>2</sub>H typically represent ~80% of the dissolved carbon present in PWW [9,59]. PPB can assimilate these VFAs present in PWW via the TCA cycle and finally convert them into cell biomass [60,61]. Although, in open photobioreactors, a fraction of the VFAs present in PWW can be lost via volatilization to the open atmosphere, VFAs assimilation in the form of PPB biomass is the main mechanism of carbon assimilation in closed systems [59]. In addition, PWW treatment can be coupled to biogas upgrading [31]. On the other hand, PPB can remove a limited nitrogen fraction with removal efficiencies of 13% to 42% from PWW via assimilation in the form of PPB biomass (Table 3). However, higher nitrogen removal efficiencies (83–99%) have been recorded in open photobioreactors due to the open nature of these photobioreactors, favouring NH<sub>3</sub> volatilization as the main nitrogen removal mechanism. Research on PWW treatment using PPB has been mainly carried out on a small scale, with pioneering research by Hülsen et al. [3] using a flat plate photobioreactor of 60, 80 and 100 L. In addition, enclosed raceways of 450 m<sup>2</sup> have been constructed within the H2020-BBI-JU project DEEP PURPLE for domestic wastewater treatment, which represents the largest PPB photobioreactor in the World.



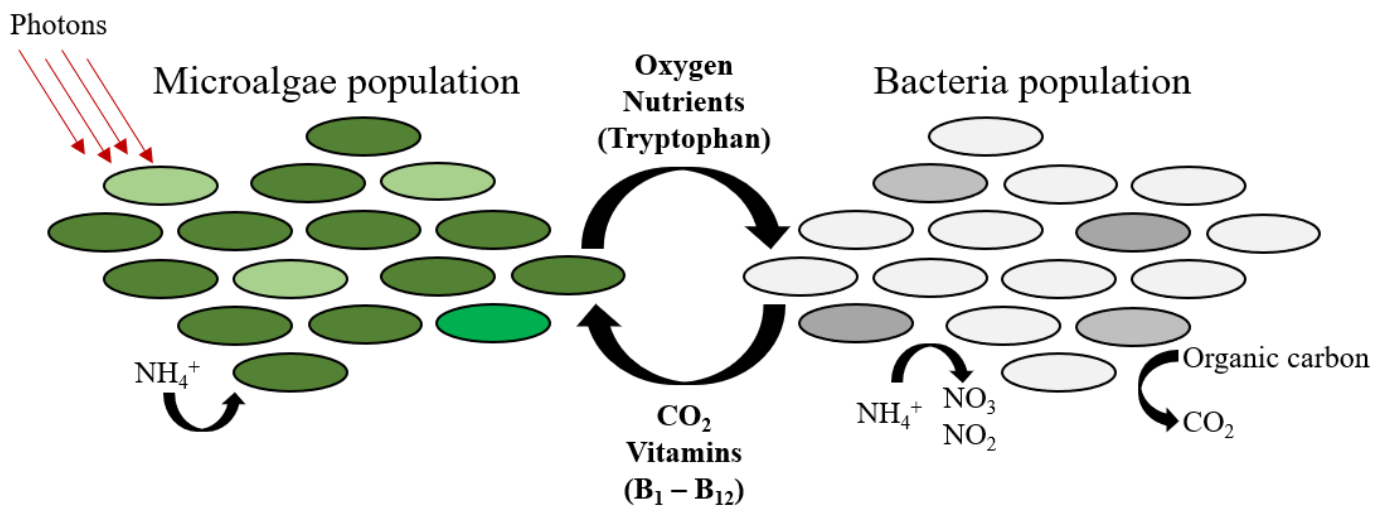
**Table 3.** Summary of piggery wastewater treatment using purple phototrophic bacteria.

PWW Characteristics (mg L <sup>-1</sup> )	Reactor Type (Volume)	Dominant Strain	Pollutant Removal (%)		References
			Carbon	Nitrogen	
TOC: 574 TN: 166	Open PBR (3 L)	<i>Rhodoplanes</i> sp.	87	83	[30]
TOC: 10,318–1989 TN: 2209–563	Batch PBR (0.4 L)	PPB mixed	78	13	[31]
TOC: 15,775–1131 TN: 5028–366	Batch PBR (0.5 L)	<i>Rhodopseudomonas</i> sp.	75	39	[59]
TOC: 1180 TN: 380	Open PBR (3 and 1.5 L)	PPB mixed	99	99	[14]
COD: 4130 TKN: 1160	Flat plate PBR (60, 80 and 100 L)	<i>Rhodopseudomonas</i> sp.	71	22	[3]
TOC: 860 TN: 380	Batch PBR (0.5 L)	<i>R. palustris</i>	79	42	[9]

#### 4.2. Microalgae-Based Treatment

Microalgae are photosynthetic microorganisms similar to plants by 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) [62]. 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 [63–66]. The main microalgae species investigated were *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum* and *Arthrospira platensis* among others. Microalgae can grow photoautotrophically, fixing CO<sub>2</sub> or photoheterotrophically by aerobically metabolizing organic compounds. Both metabolisms utilize energy from sunlight as the main energy source. Microalgae use H<sub>2</sub>O as electron donor during photoautotrophic growth to produce O<sub>2</sub> as a by-product of the reaction [39]. Indeed, this photosynthetic oxygen production is crucial to sustain life in the planet because 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 an 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<sub>2</sub> released by pollutant degrading bacteria (Figure 5) [8,67].

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) [68]. P680 catalysed the main step in oxygenic photosynthesis, where H<sub>2</sub>O is split into O<sub>2</sub> and electrons (Figure 3). 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<sub>2</sub>O. The oxidation of H<sub>2</sub>O is produced in a water-oxidizing complex catalysed by a Mn<sub>4</sub>Ca cluster. Electrons are transported through different quinones (Q<sub>A</sub> and Q<sub>Be</sub>) within P680. Those electrons are used to reduce plastoquinone (PQ) to PQH<sub>2</sub>. Electrons from PQH<sub>2</sub> are transferred to cytochrome *b*<sub>6</sub>*f*. From cytochrome *b*<sub>6</sub>*f*, 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 [68]. This photosynthesis process is noncyclic because the electrons do not return to P680 and are used for the generation of NADPH from NADP<sup>+</sup>. Nevertheless, electrons can return from PSI to PSII in some species of cyanobacteria via an electron transport chain linked (dashed line in Figure 3) [39].



**Figure 5.** Metabolic interactions between microalgae and bacteria during wastewater treatment.

The main pigments present in the photosystems of microalgae are chlorophylls, which provide them with their characteristic green colour. Chlorophyll *a* exhibits maximum absorption at 430 nm and 680 nm, whereas chlorophyll *b* absorbs mainly at 450 nm and 640 nm [39,48]. In addition, microalgae present a variety of carotenoids such as astaxanthin,  $\beta$ -carotene and lutein that absorb mainly at wavelengths between 400 nm and 520 nm (Figure 4). 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 irradiances [35,69]. 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 [5]. 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 (Figure 4).

Microalgae have a powerful photoautotrophic metabolism that allow them to grow using solar energy and assimilating inorganic compounds. Microalgae can efficiently fix  $\text{CO}_2$  via the Calvin cycle as the main carbon source [70], although are also capable of using  $\text{NaHCO}_3$  or  $\text{Na}_2\text{CO}_3$  under photoautotrophic mode. The preferred forms of nitrogen used by microalgae under photoautotrophic mode are ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) [71], which are used for the synthesis of amino acids and proteins. Some cyanobacteria species are able to fix molecular nitrogen ( $\text{N}_2$ ) from the atmosphere as the main nitrogen source. Microalgae can grow photoheterotrophically and utilize organic compounds, such as carboxylic acid, glucose and glycerol, as a carbon source, and amino acids, peptone and urea, as a nitrogen source [72]. However, PPB are more efficient than microalgae under photoheterotrophic growth and biomass production photoheterotrophically is more expensive when synthetic organic compounds are used.

Apart from nitrogen assimilation, the symbiotic association between microalgae and bacteria (Figure 5) 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 ( $\text{NH}_4^+$ ) into nitrite ( $\text{NO}_2^-$ ) and nitrite into nitrate ( $\text{NO}_3^-$ ), respectively [73]. Heterotrophic denitrification is an anoxic process performed by heterotrophic bacteria that converts  $\text{NO}_3^-$  to  $\text{NO}_2^-$  and finally to nitrogen gas ( $\text{N}_2$ ).

Microalgae in association with bacteria constitute the mainly studied 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 [74], whereas 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 [75]. An efficient treatment of synthetic food waste digestate, with removal efficiencies up to 96% and 84% for TOC and TN, respectively, was recently reported [76]. In addition, multiple studies devoted to agro-industrial wastewater treatment reporting an efficient carbon and nitrogen removal have been conducted with microalgae [8,12,30,31,33]. Typically, carbon removal efficiencies between 41% and 94%, and nitrogen removals ranging from 56% to 93% have been reported in the literature at laboratory, pilot and demo-scale (Table 4). One of the main problems associated to PWW treatment using microalgae is the inhibition by pH and high concentrations of  $\text{NH}_4^+$ , which is the main source of nitrogen present in this type of livestock wastewater. This process limitation has been recently addressed using both PPB and microalgae in sequential photobioreactors, where a significant fraction of carbon and nitrogen are initially removed by PPB. The effluent from PPB photobioreactor is treated in a microalgae photobioreactor, where microalgae favour the assimilation of the remaining nitrogen and the removal of total suspended solids [33].

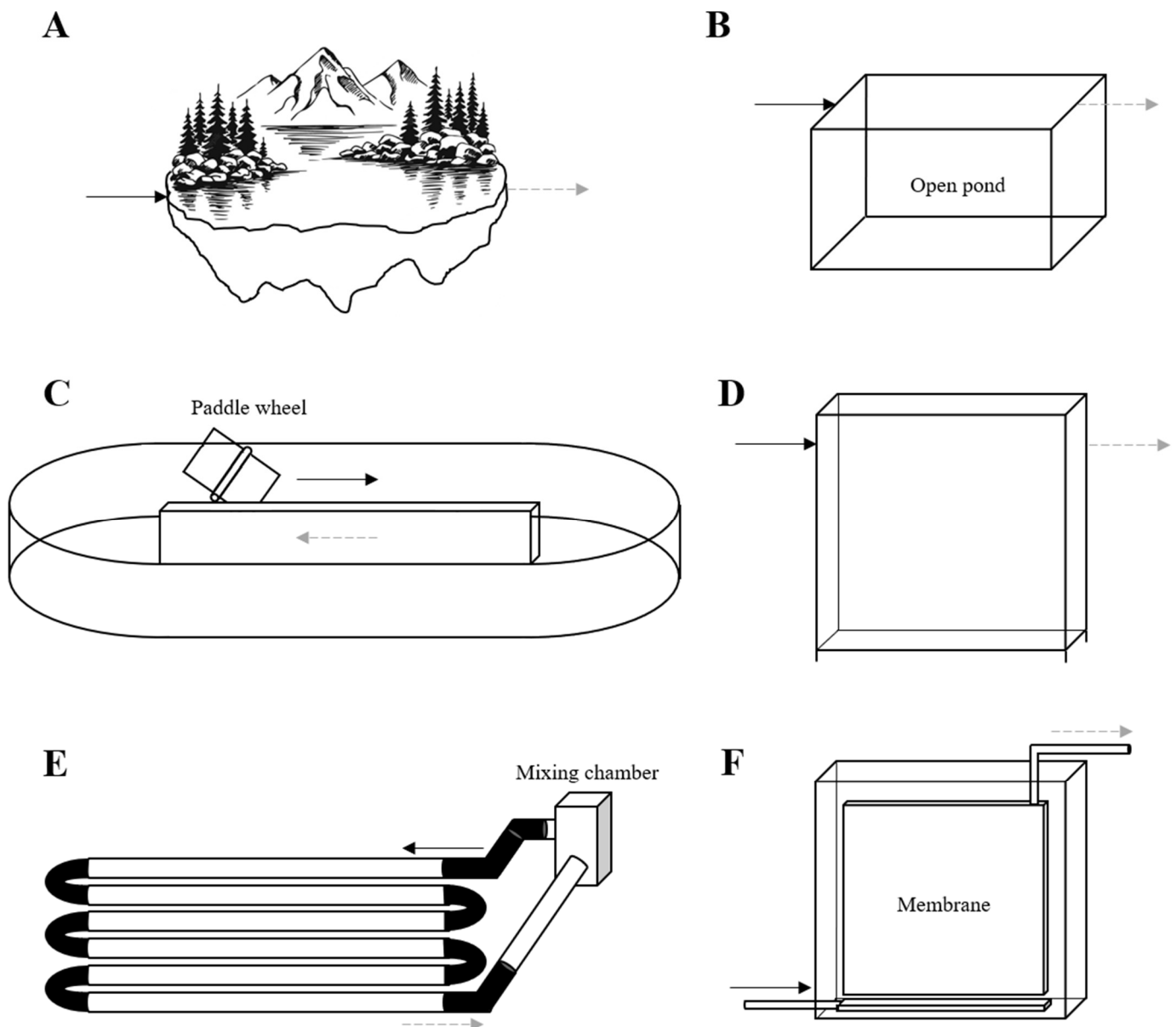
**Table 4.** Summary of piggery wastewater treatment using microalgae.

PWW Characteristics (mg L <sup>-1</sup> )	Reactor Type (Volume)	Dominant Strain	Pollutant Removal (%)		References
			Carbon	Nitrogen	
COD: 526–4346 TKN: 59–370	High-rate algal ponds (464 L)	Microalgae mixed	76	88	[8]
TOC: 963 TN: 341	Open PBR (3 L)	<i>C. vulgaris</i>	94	56	[77]
TOC: 574 TN: 166	Open PBR (3 L)	Microalgae mixed	84	87	[30]
COD: 8420–6870 NH <sub>3</sub> -N: 850–1150	Sequential Batch PBR (4 L)	Microalgae mixed	92	90	[21]
COD: 287 NH <sub>4</sub> <sup>+</sup> : 184	Membrane PBR (50 L)	<i>C. vulgaris</i>	66	74	[78]
COD: 1016 N-NH <sub>4</sub> : 92	Cylindrica PBR (10 L)	<i>Chlamydomodium fusiforme</i>	41	93	[79]

A recent study compared the removal efficiency of emerging contaminants during PWW treatment by microalgae and PPB targeting 19 veterinary drugs. Microalgae exhibited a more efficient biorremediation of oxytetracycline, doxytetracycline, enrofloxacin, sulfadiazine, tiamulin, fenbendazole, penicillin G and sulfadimidine compared to PPB. In this study, the decrease in the hydraulic retention time in the photobioreactors produced a detrimental effect in emerging contaminants degradation regardless of the photosynthetic microorganisms used [17].

## 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 [65]. 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 (Figure 6) [46]. 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 [14]. 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.

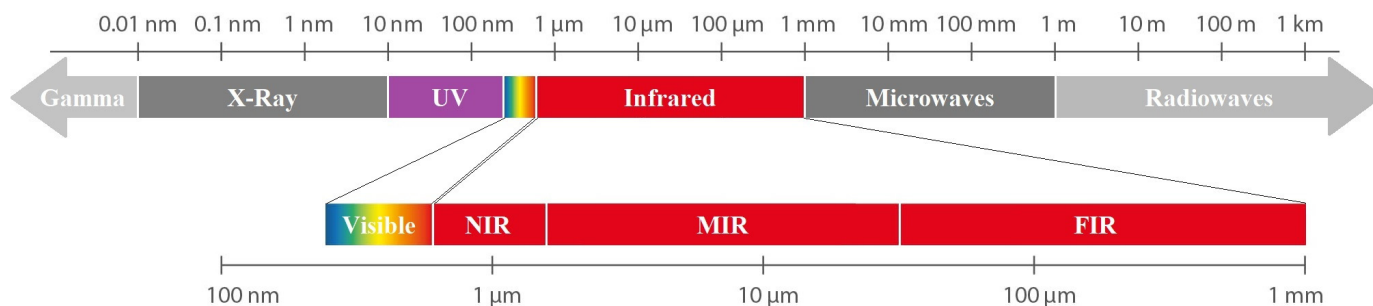


**Figure 6.** Schematic diagram of common photobioreactors for photosynthetic microorganisms cultivation. Natural lake (A), open pond (B), open raceways (C), vertical flat-panel (D), closed tubular (E) and photo-anaerobic membrane bioreactor (F).

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.

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 (Figure 4). On the other hand, PPB can absorb radiation in both visible and near-infrared (NIR) spectrum (Figure 7). 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  $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which represents the average solar intensity on a clear day [30,31].



**Figure 7.** The electromagnetic spectrum, extension of visible spectrum (400–780 nm) and infrared spectrum (780–1,000,000 nm). Infrared spectrum is composed of near-infrared (NIR: 0.780–3000 nm), mid-infrared (MIR: 3–50  $\mu\text{m}$ ) and far-infrared (FIR: 50–1000  $\mu\text{m}$ ).

Temperature is also a key parameter for cultivation of photosynthetic microorganisms. Psychrophiles are microorganisms that grow in the temperature range between 0 and 15 °C, mesophiles in temperature range between 15 and 45 °C and thermophiles are microorganisms whose growth optimum temperature exceeds 45 °C [39]. PPB and microalgae species corresponding to these three groups have been consistently reported in the literature. For instance, PPB species such as *Rhodoferrax antarcticus* have been found in the Antarctic [80], whereas the microalga *Chlamydomonas nivalis* is known to be responsible for the red colour in the snow surface [39]. In addition, an effective treatment of domestic wastewater at low temperatures (10–13 °C) using PPB has been observed [9,44,45]. Most common species of PPB and microalgae, such as *Rhodospseudomonas 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 [38]. Most studies in the 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 kill them, and oxygen tolerant, when oxygen has no biological function in their metabolism [39]. For instance, microaerophilic and anaerobic PPB can tolerate dissolved oxygen concentrations up to  $1 \text{ mg L}^{-1}$ , which are mainly used in the electron transport chain as an electron acceptor in the oxidative phosphorylation process [50]. However, oxygen can be harmful to PPB during wastewater treatment processes because the growth of aerobic chemotrophic bacteria is favoured [81,82]. On the other hand, although microalgae can grow in the presence of oxygen, very high dissolved oxygen concentrations ( $>25 \text{ mg L}^{-1}$ ) can cause damage in microalgae cells and inhibit their growth [65]. Fortunately, the oxygen produced by microalgae is rapidly utilized (as electron acceptor) by bacteria for the oxidation of organic matter and  $\text{NH}_4$  during wastewater treatment (Figure 5) [33]. In this sense, respirometric methods can be used in wastewater treatment to assess photosynthetic activity in microalgae by calculating the variation of dissolved oxygen concentration over time at a constant temperature. These respirometric methods can be used to analyse toxicity or inhibitory effects induced by wastewaters, determine their biodegradability and energy optimisation of the system to supplement oxygen when necessary [83–85].

### 5.1. Open Photobioreactors

Open ponds were the oldest configurations proposed for photosynthetic microorganisms cultivation at industrial scale for microalgae biomass production [86]. 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 [87]. 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, traditionally referred to as open raceways. 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 motorized (Figure 6) [63]. 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 irradiation), which favours the photosynthetic process. This agitation mode is simple and requires a low electricity consumption. Multiple studies describing the cultivation of microalgae and even wastewater treatment in open raceways, with moderate-to-high microalgae productivities are available in the literature [63–66,88,89]. In this context, microalgae biomass concentrations ranging from 55 mg TSS L<sup>-1</sup> in winter and up to 625 mg TSS L<sup>-1</sup> in summer have been recorded during an study that assessed the seasonal variation in a pilot scale microalgal-bacterial photobioreactor during digestate treatment coupled with biogas upgrading [89] and up to 2640 mg TSS L<sup>-1</sup> during PWW treatment in 3 L open photobioreactor [30]. 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 with biomass concentrations of up to 430 mg TSS L<sup>-1</sup> [90]. During PWW treatment in open photobioreactors, PPB biomass production nearby 873 mg TSS L<sup>-1</sup> has been described [30]. 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 [46,64].

### 5.2. Closed Photobioreactors

The design of closed photobioreactors design involves a limited contact of the cultivation broth with the atmosphere. This configuration exhibits a superior performance in terms of control of microorganisms growth, high surface area-to-volume ratio, low water evaporation losses, higher light availability and easier control of operational parameters, which increase photosynthetic biomass productivity compared to their open counterparts (Table 5) [46,86].

**Table 5.** Principal advantages and disadvantages of open and closed configurations of photobioreactors used for the cultivation of photosynthetic microorganisms.

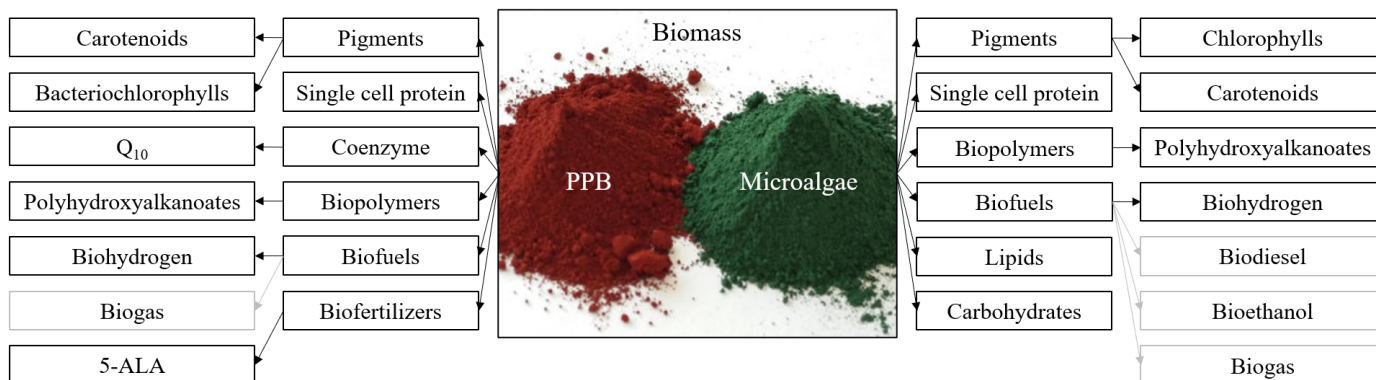
	Open Photobioreactors	Closed Photobioreactors	Reference
Capital investment	Low	High	[91]
Scalability	High	Variable	[92]
Culture control	Low	High	[64]
Culture contamination	High	Low	[64]
Evaporation rate	High	Low	[64]
Biomass productivity	Variable	High	[92]
Nutrient removal	High	High	[65]
Nutrient recovery in biomass	Variable	High	[92]

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 [65]. 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.

In recent years, the most studied photobioreactor configuration for the cultivation of PPB is the photo-anaerobic membrane bioreactor [32,45,93]. This configuration is composed of a rectangular polymethyl-methacrylate photobioreactor with a submerged flat sheet membrane (0.45 µm pore size) which is operated under anaerobic conditions (air-tight system) with internal gas recycling as an agitation strategy [93]. 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. Otherwise, high biomass concentrations of up to 920 mg TSS L<sup>-1</sup> have been recorded during PWW treatment in closed photobioreactor [33].

### 6. Biomass Valorisation

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 microorganisms into a profitable process [34,42,94]. PPB biomass contains a high content of pigments (bacteriochlorophylls and carotenoids), single-cell protein, coenzyme Q<sub>10</sub>, pantothenic acid, amino acid 5-ALA and polyhydroxyalkanoates [53]. On the other hand, microalgae can synthesize high concentrations of carbohydrates, proteins, lipids and pigments (chlorophylls and carotenoids) [26,70]. In addition, the biomass of photosynthetic microorganisms can be used as a feedstock for the production of biofuels such as biodiesel and bioethanol [95], and 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<sub>2</sub>-fixing rhizosphere and are known to accumulate compounds that are beneficial for plant growth [76,91] (Figure 8).



**Figure 8.** Summary of different potential products with high added value in photosynthetic microorganisms (PPB and microalgae). Gray frames require auxiliary processes.

**Pigments:** Photosynthetic microorganisms inherently present high concentrations and a broad portfolio of natural pigments as they are essential molecules for obtaining energy from solar radiation [68]. PPB synthesize a variety of pigments mainly of the group of bacteriochlorophylls and carotenoids, which conform to light-harvesting complexes [34]. Bacteriochlorophyll *a* and *b* are pigments found mainly PPB; both pigments have a characteristic green colour. Carotenoids provide the characteristic orange to purple colours to the group of PPB [6]. Thus, the major carotenoids synthesized by PPB are lycopene, rhodopsin, spheroidene, spirilloxanthin and their derivatives [35,96]. On the other hand, microalgae are an important source of commercial high-value compounds and can synthesize mostly pigments of the group of chlorophyll and carotenoids [69,97]. This last group of pigments include  $\beta$ -carotene, astaxanthin and lutein [97,98]. 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  $\sim 620$  nm and present promising antibacterial, anticancer, anti-inflammatory and antioxidant activities [5,99]. All those pigments are nowadays commercialized for the production of cosmetics, pharmaceuticals and functional foods.

**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. These PPB and microalgae biomass valorisation strategies are attracting and increasing attention due to the high commercial value of proteins for the production of animal feed or supplements for human nutrition [34]. PPB typically exhibits a protein content between 41% and 86% [100], whereas microalgae protein content ranges between 28% and 71% [101]. *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 [102], and as a feed supplement in livestock and aquaculture [103]. 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 ( $\text{CO}_2$  or  $\text{N}_2$ ), which represents a unique combination to reduce the production costs of single-cell protein [104].

**Coenzyme Q<sub>10</sub>:** PPB can synthesize coenzyme Q<sub>10</sub> under phototrophic conditions and accumulate similar concentrations to other microorganisms such as *Escherichia coli* and *Rhizobium radiobater* [34]. Coenzyme Q<sub>10</sub> productions from 3.1 up to 19.1 mg L<sup>-1</sup> d<sup>-1</sup> by *R. sphaeroides* and from 13.9 up to 16.4 mg L<sup>-1</sup> d<sup>-1</sup> by *R. sulfidophilus* have been previously reported in PPB species of higher coenzyme Q<sub>10</sub> production [34]. 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 Q<sub>10</sub> has antioxidant properties and is widely used in the cosmetic and medical industry [105]. Coenzyme Q<sub>10</sub> 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 [106]. These benefits for human health entail a high economical value of the coenzyme Q<sub>10</sub>, with current market prices of  $\sim$  EUR 500 per kilogram and increasing demand worldwide in the last years [34]. This coenzyme can be efficiently produced in non-toxic wastewater treatment using PPB [107].

**Polyhydroxyalkanoates:** Polyhydroxyalkanoates are intracellular biopolymers accumulated by multiple photosynthetic microorganisms and bacteria as energy and carbon reserves [108]. These molecules are a type of biopolymer with biodegradable properties and



have a high potential for substituting traditional petrochemical plastics such as polypropylene or polyethylene (produced from petroleum derivatives), which are highly harmful to terrestrial and marine biodiversity [109]. 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 [34,42]. In this context, intracellular polyhydroxyalkanoates concentrations nearby  $225 \text{ mg L}^{-1}$  have been recorded in PPB biomass [42]. Short feast periods or nutrient limitations favour the adaptation of microorganisms to accumulate polyhydroxyalkanoates over time [108]. 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 the literature for their high polyhydroxyalkanoates accumulation capacity are *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* [34]. Likewise, cyanobacteria such as *Synechococcus* sp., *Nostoc muscorum* and *Spirulina platensis* have also been described as potential candidates to produce polyhydroxyalkanoates [110]. 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.

**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 [34]. Biofuels represent a unique opportunity to boost the development of renewable energy technologies and to reduce  $\text{CO}_2$  emissions from anthropogenic activity [70].

Photosynthetic microorganisms have emerged as potential candidates for biohydrogen production due to their high conversion efficiency and the broad portfolio of substrates used to sustain their growth. Biohydrogen ( $\text{H}_2$ ) can be produced directly by PPB (*R. palustris*, *R. sphaeroides* and *R. rubrum*) mainly under a photoheterotrophic growth mode. Biohydrogen is a clean fuel as during its combustion ( $\text{H}_2 + \text{O}_2$ ) only energy and water ( $\text{H}_2\text{O}$ ) are produced [111]. This gas can be also used in fuel cells providing a very efficient alternative to combustion engines in transportation [112]. The production of  $\text{H}_2$  in PPB occurs under illumination, anoxygenic conditions and nitrogen limitations due to the synthesis of the enzyme responsible for hydrogen production (nitrogenase) is inhibited in the presence of ammonium [34,113]. Likewise, microalgae and cyanobacteria species such as *Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *Synechocystis* sp. or *Anabaena* sp. can produce directly biohydrogen [114].

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 [115]. This process includes three sequential steps, where triglycerides are converted in diglycerides, monoglycerides and finally into esters (biodiesel) and glycerol (by-product). The transesterification process involves a triglyceride and a short chain alcohol (e.g., methanol) in the 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 transesterifiable oils to produce biodiesel has not been yet investigated. Over the past 15 years, the remarkable ability of microalgae to synthesize high lipid content under nutrient limitation, with some species capable of producing up to 40–75%, has attracted significant interest and research effort for their mass cultivation as a biofuel source [63,87].

Bioethanol is a biofuel produced from the carbohydrates contained in plant biomass or microorganisms [116]. 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 [95]. 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 [117]. In addition, the CO<sub>2</sub> produced from ethanol combustion can be recovered as microalgae are capable of fixing CO<sub>2</sub>, thus neutralizing carbon emissions to the atmosphere and creating a cycling process for carbon utilization [64,118].

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 contained in these photosynthetic microorganisms occurs under anaerobic conditions in different steps catalysed by anaerobic bacteria and archaea [4]. 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<sub>2</sub> into biogas during the methanogenic step [119]. Biogas is a mixture of gases mainly composed of high concentrations of methane (40–75%), carbon dioxide (15–60%), hydrogen sulphide (0.005–3%), nitrogen (0–2%) and oxygen (0–1%) [26]. Biogas upgrading to biomethane is necessary to remove unwanted gases such as CO<sub>2</sub> and H<sub>2</sub>S if biogas is used as vehicle fuel or injected into natural gas grids. This biogas upgrading step can be carried out using PPB [27,31] and microalgae [120–122] based on the ability of these microorganisms to fix CO<sub>2</sub> and support H<sub>2</sub>S oxidation.

**Biofertilizers:** Biofertilizers are fertilizers of a biological origin that are capable of promoting growth and the nutritional quality of plants. PPB can be directly used as biofertilizers (active benefit) as these photosynthetic microorganisms can grow in symbiosis with plants in the rhizosphere and are capable of synthesizing and excreting compounds that promote plant growth. For instance, PPB can synthesize the amino acid 5-ALA, which is beneficial to plant growth [53]. 5-ALA can be used as plant growth enhancer and even possesses insecticide and herbicide properties [34,100]. 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 plants [123]. In addition, PPB biomass can be used as dried biomass (passive benefit) due to its high nutrient (N and P) content [34,91]. 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 [91]. On the other hand, the merits of microalgae biomass as slow-release biofertilizers have been consistently reported in the literature [124]. 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 [5].

## 7. Conclusions and Future Prospects

PWW management represents a serious challenge to the sustainability of pig farming due to its high production and pollutant concentrations. Conventional PWW treatments are only partially able to treat this type of wastewater at the expense of high energy consumption, prohibitive greenhouse gas emissions and a waste of resources. Photosynthetic microorganisms such as PPB and microalgae represent a promising solution for PWW valorisation due to their ability to obtain energy from solar radiation, fast growth and high nutrients assimilation. The high versatility of photosynthetic metabolism supports wastewater treatment under aerobic and anaerobic conditions in a wide variety of photobioreactor configurations. Added value bioproducts such as pigments, single-cell protein, biopolymers, biofuels and biofertilizers can be obtained from both PPB and microalgae biomass. However, despite the promising advances achieved in wastewater treatment using photosynthetic microorganisms, the future research needed to move this platform technology to commercial scale should focus on its optimization under outdoor conditions, 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 on process performance. In addition, the scale-up of continuous photobioreactors treating PWW using photosynthetic microorganisms in relevant environments and the potential of the photosynthetic biomass generated during PWW treatment must be evaluated.

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