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Review

Photobioreactor Design for Polyhydroxyalkanoate Production Using Anoxygenic Photoheterotrophs: A Review

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Abstract: This review paper provides an overview of various types of photobioreactors (PBRs) that could be used for the production of polyhydroxyalkanoates (PHAs) using anoxygenic photoheterotrophs, with a focus on the design and operation of these systems. The paper highlights the potential of different PBRs based on reactor geometry and growth mode, and also examines the advantages and disadvantages of each PBR type and summarizes their suitability for PNSB-PHA production. The optimization of reactor design and operation is crucial for maximizing PNSB growth and PHA productivity. The self-immobilization of bacteria in granular sludge is a promising technology for wastewater treatment and the production of PHAs, while grooved-surface PBRs and porous-substrate PBRs have limitations due to difficult biomass harvesting in the former and the presence of aerobic conditions incongruent with PNSB culturing in the latter. Limitations exist with all solutions for maximizing rapid growth and maintaining high biomass concentrations due to the requirements of phototrophic growth.

Keywords: purple non-sulfur bacteria; photobioreactor; polyhydroxyalkanoates; biohydrogen; biofilm

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1. Introduction

The plastic industry is one of the fastest-growing industries around the globe, and it is expected to reach a value of USD 2.19 trillion by 2022. Plastic is widely used in daily products, including electronics, transportation, medical items, textiles and packaging. Despite plastic's many benefits, including formability, light weight, strength, durability, low gas and liquid permeability and lack of conductivity, it also has a number of major drawbacks, primarily related to its inert nature, meaning it can take more than 600 years to degrade in the environment [1]. As a result, plastic waste that is not disposed properly endangers wildlife and causes solid waste accumulation and water pollution [2]. It is estimated that 51 trillion pieces of plastic are currently present in the environment. Moreover, around 6–12 million tons of plastics enter the oceans annually, posing a major threat to aquatic wildlife [3].

Bioplastics, which are derived from renewable biomass and/or biodegradable materials, can potentially provide a sustainable replacement for many petrochemical plastics. Bioplastics are made wholly or partly from biomaterials such as aliphatic polyesters, polyglycolic acids, polylactides, polysaccharides, and polyhydroxyalkanoates (PHAs). PHAs are one of the most investigated types of biodegradable biopolymers, with similar mechanical and thermoplastic properties to polyethylene and polypropylene—two of the most commonly used petrochemical plastics [4]. Despite this, PHA production on a large scale is still limited because of its high production cost as compared to petro-plastics. The price of PHA varies from 2.2 to 5.5 EUR/kg, compared to the major fossil-fuel-based plastics, which cost

less than 1.0 EUR/kg [5]. Many PHA key market players, including Danimer Scientific (Bainbridge, GA, USA), Bio-On Srl (Bologna, Italy), Shenzhen Ecomann Biotechnology Co., Ltd. (Shenzhen, China), TianAn Biological Materials Co., Ltd. (Ningbo, China), and Newlight Technologies, LLC (Huntington Beach, CA, USA), utilize pure cultures due to the higher predictability of the polymer composition and consistency of the polymer properties. This also results in two of the main costs of PHAs production—the supply of refined substrates and the costs of culture maintenance and sterilization. Therefore, in the last two decades, research has focused on developing alternative production processes to decrease PHA production costs. Such alternative processes include using low-cost substrates based on agro-wastes and wastewaters and utilizing mixed microbial cultures, requiring lower investment and operating costs [6].

Hundreds of species have been discovered to date that can synthesize PHAs. This includes a large proportion of the purple non-sulfur bacteria (PNSB), a group of anoxygenic photoheterotrophic bacteria [7]. The use of PNSB for PHA production has recently received attention because of their versatile metabolism, high substrate yield and easy enrichment in both closed and open mixed cultures, owing to their unique mode of preferred metabolism—namely, anaerobic photoheterotrophy utilizing the near-infrared light spectrum [8,9]. Open systems to support phototrophic organisms are easy to construct, operate, clean, and maintain. They have lower capital and operational costs compared to closed systems with lower energy requirements. However, open ponds, raceways and tanks require a large area for scale-up, have high evaporation losses and are susceptible to contamination and increased microbial competition—particularly in the case of anoxygenic photosynthesis. Moreover, open systems have poor biomass productivity, leading to more costly biomass harvesting [10]. Closed systems, e.g., photobioreactors (PBRs), have a number of advantages over open systems. These include better control of growth and culturing conditions with limited contamination; higher biomass concentrations; better photosynthetic efficiency; minimal water evaporation; and elimination of oxygen transfer. The major disadvantage of the PBR system is high capital and operational costs [11], creating a need to develop cost-effective PBR systems for PHA production.

Although PBRs have been widely reported and reviewed for microalgae production, the differences in metabolism between microalgae and PNSB, including the redox environment, anabolic carbon source, and light response, require a different design approach. Moreover, recent developments in microalgae PBRs have highlighted the potential for fixed-film (biofilm)-based processes as a means to reduce harvesting costs [12], which have not been adequately reported in the case of PBRs suitable for PNSB to date. Considering these points, this review focuses on the different growth factors of PNSB and triggers for PHA synthesis. It provides a detailed discussion on both suspended and biofilm-based PBRs and their suitability for PHA production using mixed cultures enriched in PNSB based on their configuration and operation modes.

2. Purple Non-Sulfur Bacteria (PNSB)

PNSB is a group of phylogenetically diverse Gram-negative bacteria belonging to the broader category of purple phototrophic bacteria (PPB) [13]. They share functional similarity and *pufLM* gene sequences with different families of Alphaproteobacteria and Betaproteobacteria. They are widely distributed in various habitats such as soil, freshwater, and the ocean and can be readily isolated from these sources [13]. Around twenty genera of PNSB are recognized [14]. The species of *Rhodopseudomonas* and *Rhodobacter* have been widely used for laboratory studies of anoxygenic photosynthesis. However, many other interesting species, some of which have at least one unusual metabolic feature, are likewise known, for example, extremophilic species inhabiting hot, cold, salty, alkaline, and acidic environments have been isolated [14].

PNSBs have a versatile metabolism [8]; therefore, they can grow under anaerobic, aerobic, autotrophic, chemoheterotrophic, and photoheterotrophic conditions [12]. They can switch between various growth modes depending on environmental conditions, including

carbon source, light source, dissolved oxygen content, pH, and C/N ratio [8,15]. Light promotes photosynthesis, while oxygen promotes respiration. Thus, the final dominant metabolism depends on the relative degrees of light, carbon and oxygen availability in the environment. For instance, in dark–aerobic conditions, PNSB performs aerobic respiration in which energy is produced by substrate-level phosphorylation and oxidative phosphorylation, and in light–anaerobic conditions, they perform photosynthesis and fermentation [16]. A summary of the features and characteristics of PNSB is provided in Table 1.

Table 1. Characteristics of PNSB, adopted from Chen et al. [17] with permission.

Aspect	Characteristics
Typical habitats	Organic polluted water (waste lagoons, sewage), soil, acidic and alkali springs, soda lakes
Photosynthetic system	Intracytoplasmic membrane
Metabolism	Photoheterotrophy (primary), photoautotroph, chemoheterotrophy
Types of bacteriochlorophylls	Bacteriochlorophylls (BChls) a and b
Form and motility	Motile, rod, spherical
Carbon source	Organic carbon, especially short-chain volatile fatty acids
Sulfur utilization	Low concentration of sulfur
Light–oxygen demand	Light–anaerobic, Light–microaerobic, Light–aerobic, Dark–aerobic, Dark–anaerobic
Preferred electron donor for phototrophic growth	Organic compounds, H_2, sulfide, ferrous iron
Salinity tolerance	0–3%
Typical bacterial genera	*Rhodopseudomonas*, *Rhodobacter*, *Rhodospirillum*

PNSB possesses purple to dark red pigments in facultative anaerobic conditions, while no pigments can be observed in aerobic conditions [8]. PNSB, in contrast to purple sulfur bacteria, only uses sulfide in a small quantity as an electron donor during photoautotrophic growth; hence the name "non-sulfur" [8,15]. For growth in photoheterotrophic mode, they acquire carbon and electrons from reduced carbon compounds. Species with the capability of growing photolithoautotrophically can use Fe^{2+}, S^{2-}, or H_2 as electron donors and CO_2 as the sole carbon source. PNSB can use different organic carbon compounds, including pyruvate, acetate, lactate, propionate, butyrate, malate and other organic acids such as amino acids [15]. Moreover, some PNSB genera can also use compounds of the C1 category as their carbon sources, such as formate and methanol. Aromatic compounds like cinnamate, phenylacetate, benzoate, chlorobenzoate, and phenol, and various carbohydrates and alcohols can also be utilized [15].

3. Polyhydroxyalkanoates (PHAs)

PHAs are a family of biologically synthesized carbon storage polymers produced from renewable resources with commercial values and similar properties to petrochemical plastics, with the additional benefit of being completely biodegradable [18]. Approximately 90 genera of microorganisms have been observed that can accumulate intracellular PHA under aerobic and anaerobic conditions. More than 150 types of PHA, with linear, branched, saturated, unsaturated, and aromatic structures, which provide different properties, have also been documented [12]. Various microorganisms produce PHAs in response to different stress conditions (such as limited phosphorus, nitrogen, sulfur, or iron) and a surplus carbon source [19].

3.1. Chemical Structure, Classification, and Properties of PHAs

PHAs are linear polyesters containing 3-hydroxy fatty acid monomers. An ester bond is established by the reaction between the hydroxyl group of one monomer and the carboxyl group of another monomer unit. Figure 1 shows the general structure of PHAs, where R indicates an alkyl group that can vary from a C1 (methyl) to a C13 (tridecyl) group. Significant variation can occur in the alkyl side chain including halogenated, aromatic, epoxidized, or other branched monomers. The obtained PHA and its molecular weight depend on various factors, including substrate, microorganism, growth conditions, and extraction. The PHA remains in an amorphous state within the cell but rapidly moves into a crystalline state during the extraction process. The alkyl group present in the general structure of PHA may ensure that characteristic properties such as biocompatibility, stereospecificity, and biodegradation exist. At the same time, the physical properties of the polymers (crystallinity, glass transition temperature, and melting point) are influenced by functional groups and the length of the side-chain molecule [12].

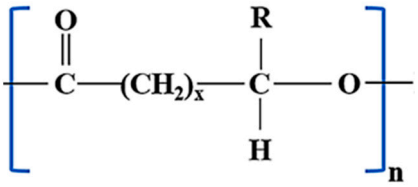


Figure 1. General chemical structure of PHAs where 'x' varies from 1 to 4 and 'n' ranges from 100 to 30,000. Adapted from Możejko-Ciesielska and Kiewisz [20] with permission.

PHAs are classified into three main groups, including long-chain-length (Lcl), medium-chain-length (Mcl), and short-chain-length (Scl) monomers, depending upon the number of carbon atoms attached. Lcl PHAs contain more than 14 carbons, Mcl PHAs contain 6–14 carbons, whereas Scl contains 3–5 carbons in their structure [21]. Lcl PHAs are less common and less studied [22]. The examples of Scl monomers include poly(3-hydroxybutyrate) (P(3HB)), poly(4-hydroxybutyrate) (P(4HB)), and poly(3-hydroxyvalerate) (P(3HV)) or the copolymer P(3HB-co-3HV). Likewise, Mcl monomer examples include homopolymers poly(3-hydroxyhexanoate) (P(3HHx)), poly (3 hydroxyoctanoate) (P(3HO)), and copolymers such as P(3HHx-co-3HO). The properties of Scl PHAs are quite different from those of Mcl PHAs (Table 2). Generally, Scl PHAs have poor tensile strength and high crystallinity. However, Mcl PHAs are elastomeric and amorphous with a low melting point and crystallinity. Ratios of both types of monomers (Scl PHAs and Mcl PHAs) in their copolymers have diverse tensile strengths and elasticities [23].

Table 2. Comparison of Scl PHAs, Mcl PHAs, and their copolymers with polypropylene, reproduced from Możejko-Ciesielska and Kiewisz [20] with permission.

Property	Homopolymer (PHAs)		Copolymer (PHAs)		Polypropylene
-	Scl	Mcl	P(3HB-co-3HV)	P(3HB-co-6%3HD)	-
Tensile strength (Mpa)	5	20	up to 690	17	400
Young's modulus (Gpa)	3.5	-	0.7–2.9	-	1.7
Elongation to break (%)	40	300	30–38	680	38
Melting temperature (°C)	179	80	137–170	130	176
Glass transition temperature (°C)	4	−40	10 to −6	−8	−10

3.2. Commercial Development of PHAs

PHAs were first observed by Beijerincka in 1888, but unfortunately, he could not define their composition and role. Later, in 1926, Lemoigne obtained PHAs from *Bacillus megaterium*. Wilkinson and Macrae proved in 1958 that PHAs in microbial cells provide a reserve of energy and carbon materials and are collected only in an increased carbon-to-nitrogen ratio [24]. Beginning in 1959, several companies started to commercialize

PHAs as environmentally friendly bioplastics. W.R. Grace and Company was the first company that tried to produce PHAs. However, they closed the company owing to the cost of PHA purification and low synthesis efficiency. After several years of struggle, in the 1980s, PHAs were produced under various trade names, including Nodax™, Biopol™, Biomer™, BioGreen™, and Biocycle™. However, the PHA market still remains limited. ADM and Metabolix, in 2006, started a joint venture—Telles, aimed to produce PHAs at large capacity, but because of uncertainty in production costs and projected capital, they closed this venture in 2012. Producers of PHAs are enthusiastic and maintain that PHAs are the new generation of biopolymers, and their market needs time to develop [20]. The PHA market is projected to experience substantial growth, with estimates suggesting that it will reach a market size of nearly USD 121 million by 2025, according to a recent market research report [25].

The main reason behind their limited production remains their high production costs, which are at least 15–17 times higher than petrochemical plastics as well as 4 to 6 times higher than marketable polylactic acid products [26]. High production costs occur due to the contributions from supplying suitable carbon substrates and high downstream processing costs [23]. The carbon substrate used for microbial PHA production can account for 45 to 50% of the total production cost [27,28]. Hence, there is a need to find efficient and cheap carbon substrates to produce cost-effective PHAs. Likewise, the downstream processing of PHAs can account for 30% of the total production cost [4] because it is challenging to recover PHAs from non-PHA cell mass (NPCM), which exists as a solid phase [4]. Several factors, including the organism-producing PHA, the required purity of PHA, its composition, and its properties should be considered while selecting a suitable PHA recovery method. For PHA recovery, various strategies have been investigated. The widely used strategy is PHA extraction using solvents such as acetone, chloroform, dichloromethane, etc. These extraction methods are hazardous to the environment and expensive as they involve extra costs for waste-solvent disposal. The enzyme digestion method for PHA recovery produces a high-quality product with a reduced environmental burden, but it is also expensive. Therefore, there is a need to optimize extraction processes to produce PHA economically [20]. Besides high production costs, the other reason for the limited success of PHAs is the low productivity of PHAs and the challenges of easily producing PHAs with specific characteristics to meet a wide range of high-value application scenarios [23].

3.3. Applications of PHAs

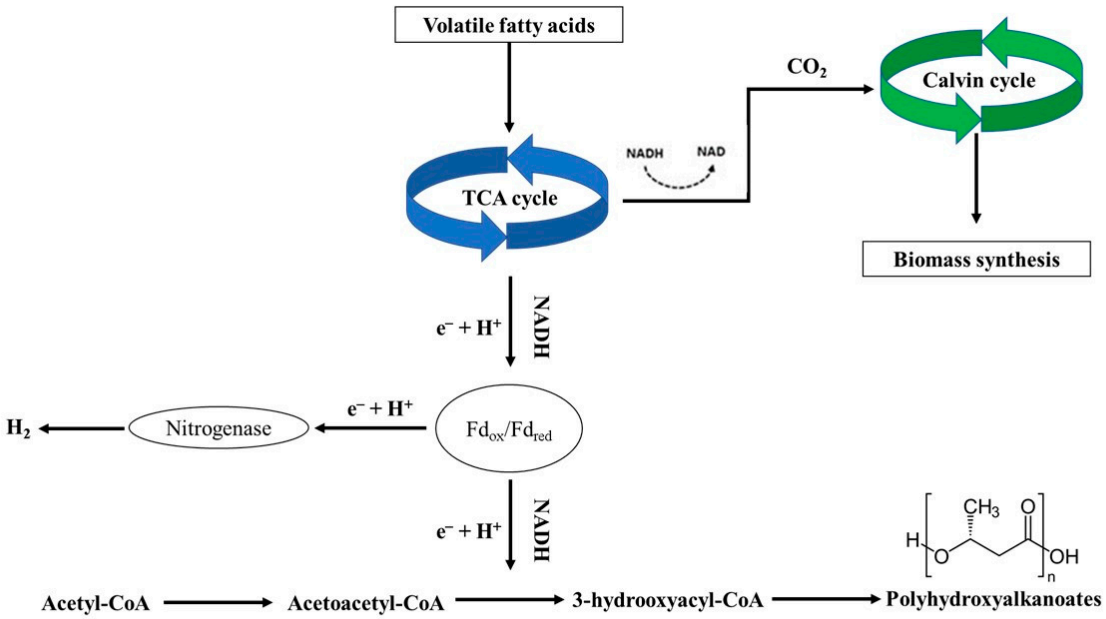
PHAs have received significant attention in recent years as consumer preferences have shifted towards biodegradable commodities. PHAs can degrade in most environments under microbial activity, including freshwater, seawater, soil, sewage sludge, and compost. Their biodegradable nature makes them suitable for a wide variety of applications. Initially, PHAs were utilized to manufacture fibers, latex, bottles, and various commercial, agricultural, or packaging materials [29]. PHAs have also been used in printer toners for printing applications and glue for coating purposes [30]. The use of PHAs in personal hygiene articles such as diapers to make them biodegradable has also been studied [31]. Currently, PHAs are widely used in the food industry, fine chemical industry, block copolymerization, the photographic industry, and the medical industry [32]. PHA eco-friendly properties, including biodegradability, biocompatibility and production from raw waste materials, aid in reducing greenhouse gas emissions and preserve limited fossil fuel resources, thus contributing to sustainable development [4].

4. PHA Biosynthesis Using PNSB

PHA production is a complicated process connected with central carbon metabolism routes. The final pathway utilized depends on the microorganism and substrate involved [33]. To date, more than ten metabolic pathways have been discovered for PHA biosynthesis. In all cases, PHA formation involves three steps, including (i) entry of a

carbon source into the cell of the microorganism through specific transporters or by passive diffusion; (ii) the metabolization of the carbon source into PHA precursors such as propionyl-CoA and acetyl-CoA, which are then transformed to (R)-3-hydroxyvaleryl-CoA or (R)-3-hydroxybutyril CoA, respectively; and (iii) the conversion of these molecules into a PHA polymer-group chain through PHA synthase [34].

Like other microorganisms, the production of PHAs using PNSB is one of the many ways to dispose of excess reducing equivalents and reach redox equilibrium. They absorb light energy (photons) through their photosystem and store it as chemical energy (Adenosine triphosphate-ATP), which is required for cell growth and cell function. Volatile fatty acids are among the most easily assimilated substrates by PNSB and are oxidized into H^+, CO_2, and electrons through the tricarboxylic acid cycle (TCA). When biomass production is not possible (through CO_2 fixation via the Calvin cycle), these bacteria dissipate excess electrons via PHA and hydrogen production, as presented in Figure 2 [12]. This is frequently associated with nutrient limitation, in particular nitrogen limitation—as is common for many organisms that produce PHA. However, PNSBs are also capable of producing PHA under concomitant growth, particularly with excess irradiation, such that the electron generation exceeds the ability to simultaneously build biomass [35].



TCA: Tricarboxylic acid, **NADH:** Nicotinamide adenine dinucleotide + Hydrogen, **NAD:** Nicotinamide adenine dinucleotide, e^-: Electron, H^+: Hydrogen ion, $\mathbf{Fd_{ox}}$: Ferredoxin oxidation, and $\mathbf{Fd_{red}}$: Ferredoxin reduction.

Figure 2. General scheme of the metabolic pathways involved in PHA, biomass, and hydrogen production using PNSB. Adapted from Montiel-Corona and Buitrón [12] with permission.

4.1. Influence of Carbon and Nutrients

Quantitative studies on PHA metabolism using phototrophic PNSB are limited only to a few species, such as *Rhodobacter sphaeroides* [36,37], *Rhodospirillum rubrum* [38–40], *Rhodopseudomonas palustris* [41], *Afifella marina* [42], *Rhodopseudomonas julia* [43,44], *Rhodopseudomonas* sp. S16-VOGS3 [45,46], *Rhodovulum tesquicola*, *Roseovarius goensis*, and *Roseovarius visakhapatnamensi* [47]. PNSB-PHA production depends on various physicochemical and operational parameters, including carbon (C) and nitrogen (N) source, C/N ratio, phosphorous (P), sulfur (S) and magnesium (Mg) concentration, pH, temperature, light intensity, wavelength, and light/dark cycle. Sources with high carbon content are suitable for producing more PHA [48,49]. Fatty acids, specifically butyrate and acetate, favor PHA production [50]. However, substrates rich in sugar and starch are not recommended for PHA because they promote H_2 production. Touloupakis et al. [46] used different carbon sources (malate, succinate, and acetate) and glutamate (nitrogen source) for PHA produc-

tion using *Rhodopseudomonas* sp. They found acetate a promising source for high PHA production (69 mg·L^{-1}) and an 18.3% dry cell weight (DCW) [46]. Likewise, another study using a *Rhodobacter sphaeroides* strain on various nitrogen and carbon sources found the highest PHA content of 40% DCW with ammonium and acetate [37]. However, Carlozzi and Touloupakis [51] found lactate to be the best carbon source, followed by acetate and butyrate, with ammonium and glutamate as a nitrogen sources for higher PHA production using *Rhodovulum sulfidophilum*. Hence, it can be concluded that acetate is generally a preferred carbon source, but the best source may vary depending on the strain or genus of PNSB, while glutamate and ammonium work well as nitrogen sources. Both lactate and acetate are widely produced as a byproduct of various industries or by fermentation. Therefore, many wastewater streams may be applicable with a fermentation reactor rather than a photobioreactor.

The absence or limitation of nutrients (N, P, S, and Mg) used for cell development and anaerobic conditions have been shown to result in higher PHA production [52]. C/N ratios above 30 are suggested [48]. Carlozzi et al. [53] investigated PHA production by culturing *Rhodopseudomonas* sp. S16-VOGS3 under different nutrient-deficient conditions, using nitrogen, phosphorous and sulfur. They found higher PHA accumulation in sulfur-deficient conditions and reported it as the best strategy for producing PHA-rich biomass. Sulfur-deficient conditions cause the inhibition of nitrogenase activity and ultimately hydrogen production and a concomitant enhancement in PHA production [53].

Heavy metals such as Cu, Mn, Fe, and Zn affect the PHA production yield and, therefore, their levels must be low [54]. Relatively neutral pH values of approximately 6.5 [55] and 7.0 [56] have also been recommended. However, few authors reported slightly alkaline pH-enhanced PNSB-PHA production, as hydrogen production was favored at lower pH levels [37]. Likewise, the temperature for fermentation should be maintained between 24 and 30 °C [50]. PHA production can be achieved using both pure and mixed cultures of PNSB [55]. The data so far indicate that pure cultures produce more PHA with a higher yield than mixed cultures but have a higher PHA production cost [57]. Therefore, PNSB-PHA production using a mixed microbial culture is widely used [50].

4.2. Influence of Light

It has been reported that light sources, light intensity, light wavelength, and photoperiod impact PSNB growth [58]. This is directly linked to the light-harvesting apparatus of PNSB. Photosynthetic bacteria contain carotenoid (Crt) and bacteriochlorophyll (BChl) pigments situated in the light-harvesting complexes [59,60]. Crts absorb light in the blue-green spectral region of visible light, whereas BChls show two bands, including the Q band in the visible region of the spectrum and the Soret band in the near-UV region (around 390 nm, not shown in Figure 3). The Q band is further decomposed into Q_x and Q_y bands based on their predominant polarization. Additionally, BChl absorption peaks in the near-infrared region (800, 850, and 880 nm) result from the Q_y shift. The peaks at 880 and 850 nm usually combine in one prominent peak, with the highest absorbance at an intermediate wavelength in between, but shoulders can rarely be seen. The peak at 590 nm is because of BChl and $-Q_x$ band shifting. Furthermore, carotenoids are accountable for shifting electronic excitation to BChls, after which the separation of charge occurs [61].

When purple bacteria are cultured under low light intensities, light-harvesting complexes (LHCs) increase in quantity to better harvest the available number of photons effectively [60,63]. On the other hand, when purple bacteria are grown in high light intensities, the number of LHCs decreases to prevent photodamage [64]. During the light-harvesting mechanism, the peripheral LHCs, i.e., LH2 [63,65], are the first structure for trapping light (energy) which then transfers energy to the core complex LH1-reaction center where charge separation occurs.

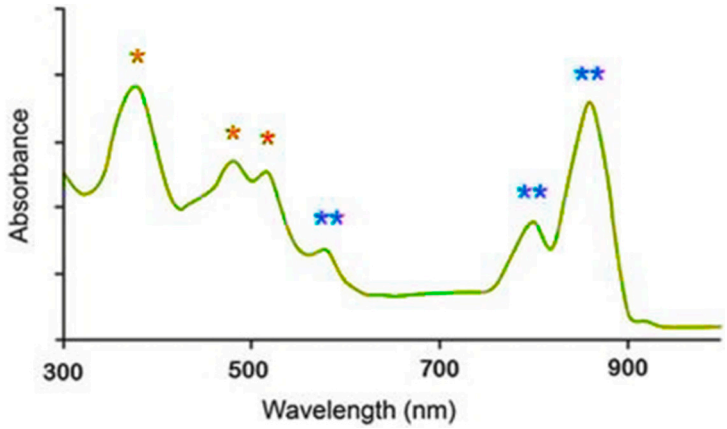


Figure 3. Typical absorption spectrum of PNSB. * shows absorption peaks of Crts, while ** represents absorption peaks of BChls. Reproduced from Adessi and De Philippis [62] with permission.

PNSB can use a wide range of the sunlight spectrum, and about 65.8% of photosynthetic active radiation is available for purple bacteria [66]. However, one issue with solar light is the intrinsic variability, leading to the varying light rate and intensity during the daytime, which ultimately results in varying biomass and biomolecule production rates. Additionally, the highest irradiation at noon (around 900 $W \cdot m^{-2}$) can cause photoinhibition [67]. Therefore, to increase the overall duration of light exposure, the supplementation of artificial lights can be used [62].

Various artificial light types have been used to culture PNSB. Tungsten lamps are popular because their light emission spectrum matches the PNSB absorption spectrum (Figure 4), specifically, the emission of near-infrared wavelengths, where the absorption maxima of bacteriochlorophylls are situated. However, tungsten filament lamps are energy-intensive light sources as they emit a significant proportion of energy as heat. Therefore, light-emitting diodes (LEDs) have gained popularity as an alternative [62]. The lifetime of LEDs ranges from 20,000 to 30,000 h, whereas a tungsten lamp has a lifetime of about 1000–2000 h. Moreover, using LEDs instead of tungsten lamps can reduce energy costs by 98% [60,68]. Incandescent and halogen lamps are also frequently used [62]. Infrared light as an energy source, and the use of infrared light as a light source for PNSB growth, is still an area of active research. However, for scaled-up systems, the most cost-effective source of light will be natural light, i.e., sunlight [68], because a significant portion of the expenses in photobioreactor systems is attributed to the provision of artificial lighting [69].

In general, the use of infrared (IR) light can enhance PHA production in photobioreactors by selectively promoting the growth of PNSB. IR light is absorbed by PNSB, but not by many other bacteria, allowing for selective enrichment of PNSB in mixed cultures [70]. This strategy can be beneficial at the laboratory scale to maximize PHA production. Moreover, maximizing light capture is crucial for all photobioreactor designs, particularly for larger-scale operations. The panels of the PBR should be angled towards the sun to maximize solar light capture. The optimal angle can depend on the latitude and season of the location. Further, the use of reflective surfaces or mirrors can increase the amount of light that is reflected onto the panels, enhancing light capture. These modifications can increase the efficiency of PHA production by increasing the amount of light available for PNSB growth.

Light plays an important role in PNSB growth but increased biomass concentration, pigments and PHA production can result in light attenuation in the PBR. Capson-Tojo et al. [71] investigated the effect of reactor configuration, biomass, pigments, and PHA production on the light attenuation in a PPB-enriched culture. The attenuation extent of ultraviolet-visible and near-infrared light was investigated. They found that a flat-panel PBR was better as compared to a cylindrical PBR in terms of light attenuation. The increasing concentration of biomass results in higher light attenuation, and the concentration of Crts and BChls have strong effect on light attenuation. For instance, on average, in dense PPB cultures with biomass concentration $\geq 1000\times$ g $COD \cdot m^{-3}$, the effective light penetration is only

5 cm. The PHA content in cells did not affect light attenuation significantly. The findings of this study suggested that while designing PBRs, light attenuation must be taken into consideration.

A recent study investigated the impacts of different light sources (i.e., halogen lamp-HL, incandescent lamp-IL, IR, and LEDs of white, red, blue, green, and yellow) on the production of biomass, pigments, and protein by *Rhodobacter sphaeroides*. The results suggested that the incandescent lamp was the optimal light source for the production of biomass and protein [72]. Likewise, Kuo et al. [60] used four different light sources for *Rhodopseudomonas palustris*, studying growth and carotenoid production after 144 h of cultivating time. Light sources used were HL, FL, IL, and LEDs. Among all light sources, blue LED light showed higher biomass production, while yellow LED light showed the least biomass production. Likewise, concerning energy efficiency for bacterial growth, blue and white LEDs were found to rank first and second, respectively, while FL and HL were found in sixth and seventh place. Moreover, carotenoid productivity was found highest with blue LED light, followed by yellow LED, white LED, green LED, IL, HL, red LED, and FL.

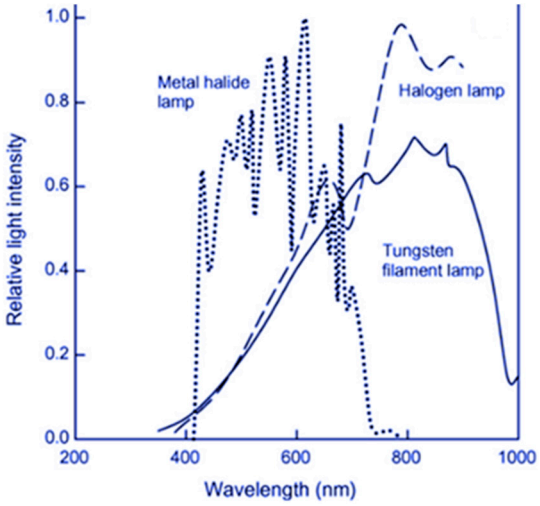


Figure 4. Light-emission spectra of halogen lamp (---), metal halide lamp (⋯) and tungsten filament lamp (solid line). Reproduced from Chen et al. [73] with permission.

Zhou et al. [74] conducted a similar study investigating biomass and pigment production and pollutant removal from wastewater under various light sources using a *Rhodopseudomonas* strain with different light sources at a light intensity of 2000 lx. An incandescent lamp and four different-colored LEDs (blue, yellow, red, and white) were used. Each light source was used with a specific wavelength (red: 650 nm, yellow: 595 nm, blue: 479 nm) except the white LED and incandescent lamp. Moreover, 18 W power was used for red, yellow, and blue LEDs, while for white LED and the incandescent lamp, 9 W and 80 W power were used, respectively. Zhou et al. [74] found red LED to be the best light source for biomass production, ATP production and COD removal. The yellow LED produced more pigments, while the red LED produced a higher Crts/BChls ratio. These results differed from those of Kuo et al. [60], who performed a similar study. The contradiction between studies may be due to the strain used. Zhou et al. [74] did not mention the exact strain of *Rhodopseudomonas*, whereas Kuo et al. [60] conducted their study on *Rhodopseudomonas palustris*. Additionally, substrate and culture duration were also different. Nevertheless, the two studies indicate that wavelength can play a critical role in system performance.

According to Muzziotti et al. [75], the use of halogen lamps showed that *Rhodopseudomonas palustris* synthesized a PHB content of 14.7% under high-light conditions (1500 µmol photons $m^2 \cdot s^{-1}$) as compared to 12.2% under low-light conditions (250 µmol photons $m^2 \cdot s^{-1}$). On the other hand, Higuchi-Takeuchi and Numata [76] found that when using LED (800 nm) lights, *Rhodovulum sulfidophilum* produced a higher PHA con-

tent of 17–50% under low-light conditions (8 $W \cdot m^{-2}$) compared to 15–30% under high-light conditions (50 $W \cdot m^{-2}$). One of the main reasons for the difference in PHB content between the two studies is the difference in the species of bacteria used, since *Rhodopseudomonas* and *Rhodovulum sulfidophilum* may have different metabolic pathways and responses to light. Another factor that could contribute to the difference in PHB content between the two studies is the type and intensity of the light source. LED lights typically emit a narrow-spectrum light, which may be more favorable for PHB production compared to the broad-spectrum light emitted by halogen lamps. Additionally, the specific spectral distribution and intensity of the LED lights used in the study by Higuchi-Takeuchi et al. [76] may have also influenced the difference in PHB content.

In addition to the type of light source, the intensity of light, and the wavelength of light, the duration of light exposure, known as the photoperiod, is a crucial factor in PNSB biomass and PHA production. Montiel Corona et al. [77] discovered that shorter dark/light cycles of 30 min/30 min were more favorable for PHA production. However, they also found that the shortest photoperiod of 15 min/15 min resulted in slightly lower PHA production for *Rhodobacter capsulatis*. On the other hand, some studies have shown that dark conditions can stimulate PHA synthesis due to the TCA cycle and nitrogenase-activity limitation in dark conditions [55]. Fradinho et al. conducted an experiment and found that under a 4 h light/4 h dark cycle, the anoxygenic phototrophic bacteria (APB) doubled its net PHA accumulation rate and increased its overall PHA accumulation to 30% PHA/VSS in comparison to continuous light availability [78,79]. Another study reported that PHA contents were higher in anaerobic–light culture as compared to aerobic–dark culture. However, the growth rates were lower in anaerobic–light culture, and the PHA concentrations in the culture were also low [80] in five PNSB strains out of the seven strains tested. Hence, the optimal photoperiod for PHA production varies between different PNSB strains and can be influenced by factors such as the type and concentration of the carbon source, the presence of other nutrients, and the temperature of the culture.

5. Photobioreactors

Photobioreactors are reactors that can pass light through their transparent wall and carry out biological processes [81]. PBRs are highly productive compared to open-air systems like raceway ponds and high-rate algal ponds [82]. They result in less water evaporation and long-term culture maintenance and can support higher volumetric cell densities [83]. One of the main design considerations for PBRs is the effective utilization of light [62]. Light distribution, light sources, light quality, and intensity are crucial for both biomass growth and PHA accumulation using PNSB [45]. When light availability is less than a certain threshold, it results in switching PNSB metabolism to unfavorable metabolic modes such as dark fermentation. This can be avoided by designing PBRs with a high surface-to-volume (or length-to-thickness aspect) ratio [84]. This is a tradeoff, however, as PBRs with a very high surface-to-volume ratio require more land area and cost, ultimately leading to complications in PBR design and operational issues [85].

The light used in PBRs for PNSB growth can be artificial or natural, or a combination of both, as discussed in Section 4.2. Lighting can also be provided internally to the reactor using various optical technologies. Material selection is also essential to capture maximum light. Various materials exist for the construction of PBRs, including polyvinyl chloride (PVC), polyethylene, acrylic PVC, plexiglass, and glass [86]. Glass is the most transparent of these materials, and low-density polyethylene is the least transparent. Glass is suitable for PBR construction in terms of permeability and has good mechanical strength and durability [87]. However, large-scale PBR construction needs many connection parts that could be costly, since glass is brittle. Microbial adhesion to the selected PBR surface material is another important aspect that should be considered while designing a PBR since it decreases the penetration of light inside the PBR [86].

Mixing is a necessary process in PBRs to reduce nutrient gradients, increase mass transfer, and enable the separation of the produced gas and liquid culture. It avoids cell

sedimentation [88] and allows cells to receive a more uniform intensity of light [62]. Thus, for a very efficient biological process, it is important to either spread out the light evenly throughout the reactor (dispersing light), reduce the concentration of light in any one area by spreading it over a larger volume (diluting light), or ensure that the cells are frequently exposed to areas of higher light intensity through proper mixing [66].

Thus, an efficient PBR should have the following characteristics: (1) the capability of harvesting and transporting light as much as possible, and distributing light in a manner such that most of the light energy is used for biomass formation; (2) permit the precise and appropriate control of operational parameters, which aids bacterial cells in growing and using light energy efficiently; (3) reduce capital and operational costs; and (4) decrease energy consumption during the process [86]. Other critical factors include mass transfer and the control of substrate/nutrient concentrations, ease of maintenance and operation, and environmental compatibility.

PBRs, like most bioreactors, can be divided into suspended systems, immobilized systems, and fixed-growth biofilm systems. In the literature, various studies reported different PBRs that utilized PNSB for wastewater treatment and hydrogen production. However, only a few studies reported PHA production (Table 3).

Table 3. Summaries of different PBR studies with PNSB and some microalgae where PNSB studies are limited.

PBR	Purpose	Strain	Illumination	Scale	References
Flat-panel (plate) PBR	Hydrogen production	*Rhodobacter sphaeroides* (O.U. 001)	Artificial	Laboratory-scale	[89]
	Hydrogen and PHB production	*Rhodobacter sphaeroides* (O.U. 001)	Solar	Laboratory-scale	[90]
	PHB production	*Rhodopseudomonas sphaeroides* S16-VOGS3	Solar	Laboratory-scale	[45]
	PHB production	*Rhodopseudomonas palustris* 42OL	Solar	Laboratory-scale	[91]
Tubular PBR	Biomass production	*Rhodopseudomonas palustris*	Artificial/Solar	Laboratory-scale	[92]
	Hydrogen production	*Rhodopseudomonas palustris*	Solar	Laboratory-scale	[93]
	Hydrogen production	*Rhodobacter capsulatus*	Solar	Pilot-scale	[81]
Membrane PBR	Wastewater treatment	Microalgae *(Chlorella vulgaris)*	Artificial	Laboratory-scale	[94]
	Wastewater treatment	*Rhodopseudomonas palustris*	Artificial	Laboratory-scale	[95]
Up-flow anaerobic sludge blanket (UASB) PBR	Wastewater treatment and PHB production	Mixed culture of phototrophic bacteria	Artificial	Laboratory-scale	[96]
Artificial immobilization system	Hydrogen production (gel)	*Rhodopseudomonas palustris* CQK 01	Artificial	Laboratory-scale	[97]
	Hydrogen production (agar)	*Rhodobacter capsulatus* (DSM 1710 wild-type strain) and YO3 (Hup-mutant of MT1131 strain)	Artificial	Laboratory-scale	[98]
	Hydrogen production (silica gel, clay and activated carbon)	*Rhodopseudomonas palustris* WP3-5	Artificial	Laboratory-scale	[73]
Moving-bed Biofilm PBR	Wastewater treatment	NA [1]	NA	Laboratory-scale	[99]
	Wastewater treatment	*Marine macroalgae*	Artificial	Laboratory-scale	[100]
Photo-rotating biological contactor	Wastewater treatment	*Anoxygenic photosynthetic bacteria*	Artificial	Laboratory-scale	[101]
Porous-substrate PBR	Astaxanthin production	Microalga *Haematococcus pluvialis*	Artificial	Laboratory-scale	[102]
Grooved-surface PBR	Hydrogen production	*Rhodopseudomonas palustris* CQK 01	Artificial	Laboratory-scale	[103]
Flat-panel (plate) biofilm PBR	Protein production	Mixed culture of purple bacteria	Solar	Pilot-scale	[104]
Pipe-overflow recirculation biofilm PBR	Verification of mathematical model for substrate consumption and biofilm productivity	Mixed culture of photosynthetic bacteria	Artificial	Laboratory-scale	[105]

[1] Not applicable.

5.1. Suspended-Culture PBRs

In suspended-culture PBRs, microbial cells grow as a planktonic form in a bulk liquid medium without any support to the substratum [106]. Suspended systems permit good mass transfer between substrates and microorganisms [97] but have issues sustaining a satisfactory level of bacterial cells at short hydraulic retention times (HRT) because of biomass washout. The suspended system requires biomass recycling to acquire sufficient cell density, long HRTs or to be operated in batch mode [98,107]. PNSBs exhibit different growth rates under various growth modes, including photoautotrophic, photoheterotrophic, chemoautotrophic, and chemoheterotrophic conditions. The exact growth rates and doubling time depend on the species of PNSB and the specific environmental conditions, such as carbon source, temperature, light intensity, and nutrient availability. For instance, *Rhodopseudomonas capsulate* B10 doubling time was found to be 3.5 h under photoautotrophic (anaerobic) metabolism and 1.8–2.0 h under photoheterotrophic metabolism with lactate and malate as carbon sources. Under chemoautotrophic (aerobic and dark) metabolism, the doubling time was 6.0 h, and under chemoheterotrophic (aerobic and dark) metabolism, it was between 1.8 and 2.8 h with lactate and malate as carbon sources [108]. Therefore, it is likely that a relatively short HRT (5–17 h) would be sufficient to maintain a high biomass concentration of PNSB in a PBR. However, it should be noted that the optimal HRT can vary significantly among different species and is closely linked to the specific growth rate (μ) of the organisms. For a PBR study on one type of microalgae grown on wastewater, a theoretical relationship between the optimal HRT for biomass productivity and the specific growth rate was given as HRT = 2/μ [109]. Further study is required to determine if such relationships hold true for PNSB, given the differences in light penetration of their preferred light spectra (i.e., near-infrared) and their different preferences for growth mode (i.e., photoheterotrophic).

In this section, suspended PBRs reported in the literature for PNSB-PHA production are discussed. They are classified into three main types, including flat-panel, tubular, and membrane PBRs. Each suspended-system PBR has different advantages and disadvantages, which are summarized in Table 4.

Table 4. Advantages and disadvantages of suspended PBR systems and their relevance to PNSB-PHA production.

Suspended PBR	Advantages	Relevance to PNSB-PHA Production	Disadvantages	Relevance to PNSB-PHA Production
Flat-panel	1. High area-to-volume ratio 2. Comparatively economical 3. Can be constructed and modified easily 4. Partial plug flow	1. Applicable: More efficient light exposure to generate excess, reducing equivalents directed to PHA 2. Applicable: Produce cost-effective PHA 3. Applicable: More efficient light exposure to generate excess reducing equivalents directed to PHA and higher average growth rates 4. Applicable: Allows higher rates of growth and development of spatial nutrient variation	1. Difficult to scale up 2. Limited mixing in very-thin flat-panel PBRs	1. Moderately applicable: Difficulty in large-scale production of PHA. As an emerging industry, its modular scale-up may be beneficial 2. Applicable: Low productivity and PHA yields. May result in biofilm formation on walls
Tubular	1. Large available surface area for illumination 2. Practicable and scalable (horizontal orientation) 3. Plug flow	1. Applicable: Making them well-suited for PNSB-PHA production 2. Applicable: Making it a suitable option for industrial-scale PNSB-PHA production 3. Applicable: Allows higher rates of growth and development of spatial nutrient variation	1. High energy requirements 2. Wall cleaning maintenance issue 3. Photo-limitation	1. Applicable: Can increase the production costs 2. Applicable: Can increase operational cost and reduce productivity. More difficult to clean than flat panel 3. Somewhat applicable: May impact growth rate but is beneficial to drive PHA synthesis. As PNSB are mixotrophic, the diameter could possibly be enlarged

Table 4. *Cont.*

Suspended PBR	Advantages	Relevance to PNSB-PHA Production	Disadvantages	Relevance to PNSB-PHA Production
Membrane	1. Highly concentrated biomass 2. Independent control of solid retention time (SRT) and hydraulic retention time (HRT) 3. Workable at low HRTs	1. Partially applicable: Membrane PBRs have high biomass concentration and are beneficial for harvesting but will limit light penetration 2. Applicable: Provides greater operational flexibility and optimization for maximum PHA production 3. Applicable: Allows a compact reactor	1. High biomass-creation rates need low biomass concentration to avoid clogging or blocking of the system 2. Membrane fouling	1. Applicable: High biomass concentration is often required for efficient PHA production 2. Less applicable: The lower extracellular polymeric substance (EPS) production of PNSB should reduce membrane fouling issues

5.1.1. Flat-Panel (Plate) Photobioreactors

Flat-panel PBRs are rectangular with two parallel sides of large surface area that are placed either inclined in the direction of the sun or vertically (Figure 5a). These PBRs can be constructed and modified easily. They are highly suitable for mixing and light distribution [62], are economical compared to other PBRs and have a high area-to-volume ratio at a minimum thickness [89]. The main issue with these PBRs is the difficulty in scale-up because of their many compartments and support materials. However, there is the possibility to enhance ground-area productivity by arranging a set of reactors adjacent to each other (parallel) at a suitable distance. The thickness of flat-panel PBRs is generally in the range of cm(s) [62]. However, the optimum thickness depends on various factors, including mixing, light intensity, and biomass culture.

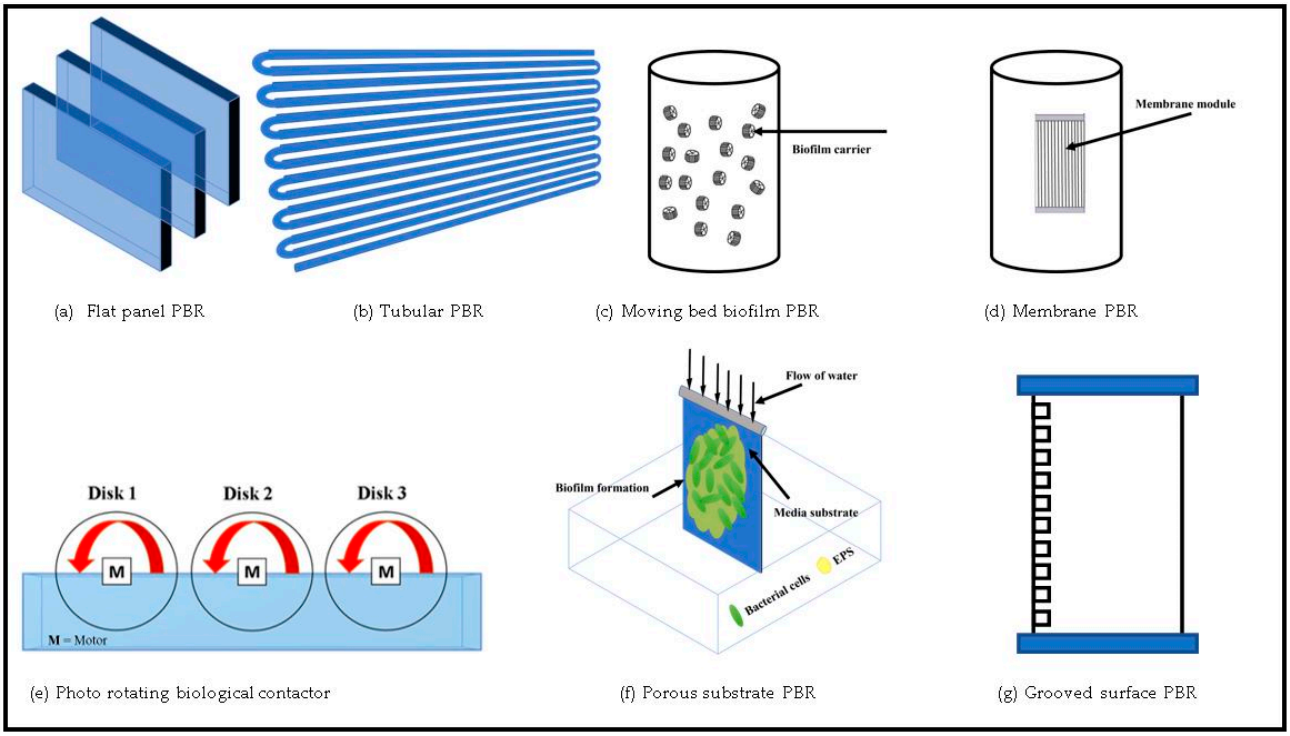


Figure 5. Schematic diagrams of different PBRs.

Flat-panel PBRs can present certain challenges due to their design. One key issue is that the close spacing of the walls may lead to laminar rather than turbulent flow. This can restrict the mixing of the culture, making it difficult to evenly distribute nutrients and light, which are crucial for the growth and metabolic activities of photoheterotrophic organisms. This limited mixing can have a detrimental effect on productivity, especially during the

scaling-up of these PBRs [89]. Various methods are available to overcome agitation limitations, including impeller stirring, baffles, sparging, gas recirculation, magnetic stirring, and rocking motions. All agitation methods require energy [110], which can increase the production costs of PHAs. Gilbert et al. [89] developed and enhanced a flat-panel PBR by incorporating a rocking motion, increasing the frequency of light exposure and substrate mass transfer. They used *Rhodobacter sphaeroides* O.U. 001 strain, and a rocking motion resulted in 3.3% better light conversion efficiency, reaching 44.4% substrate (malic acid) conversion efficiency, and 11 $mL \cdot L^{-1} \cdot h^{-1}$ maximum hydrogen production [89].

Flat-panel PBRs studies on PNSB-PHA production are rare, and much research is needed to study them for PHA production as most studies reported in the literature focus on hydrogen production [89,107,111]. However, Eroglu et al. [90] developed an 8 L temperature-controlled flat-panel solar PBR for PHA, carotenoid, and hydrogen production by utilizing *Rhodobacter sphaeroides* O.U. 001. The flat-panel PBR utilized in the study was made of plexiglass of 5 mm thickness with a 0.2 m^2 illuminated front area. They found a maximum PHA accumulation of 5.4 $mg \cdot g^{-1}$ wet weight of bacteria, with a productivity of 0.45 $mg\text{-}PHB \cdot d^{-1}$, using various carbon substrates.

Flat-panel PBRs can be effective for PHA production, which can be utilized at both the laboratory scale and on a larger scale with certain modifications. In this regard, several modifications can be incorporated to improve the efficiency of PHA production and maintain optimal environmental conditions for the growth of the PNSB. For instance, temperature-control systems can be used to avoid overheating but will increase the overall cost of the system [91]. To avoid the formation of a biofilm on the panels, which can decrease light penetration and PHA production, the biofilm must be removed. Mechanical agitation or stirring can increase the mixing of the culture and avoid the formation of a biofilm, promoting even nutrient distribution and enhancing PHA production.

5.1.2. Tubular Photobioreactor

Tubular PBRs are advanced systems designed for photosynthetic bacteria, including PNSB, in a controlled environment. They are suitable for outdoor mass cultivation as they possess a large available surface area for illumination [86,112]. They consist of long, circular transparent tubes that allow for maximum light penetration and provide the energy required for photosynthesis (Figure 5b). The tubes that are generally used are 10–60 mm in diameter and several meters long [113]. The tubes can be constructed from materials like glass, plastic, or polymer, and can be arranged in various patterns (e.g., straight, bent, or spiral) and orientations (e.g., inclined, helical, vertical, and horizontal) [114]. They are widely used for large-scale biomass production for a variety of purposes, including biofuel production, wastewater treatment, and the production of high-value compounds like bioplastics, pigments, enzymes, and pharmaceuticals. The transparent tubes allow for more efficient light penetration, resulting in higher photosynthetic activity and improved biomass productivity. The closed, controlled environment of tubular PBRs also allows for precise control over factors such as temperature, pH, and nutrient levels. Additionally, tubular PBRs reduce the risk of contamination from external sources, making them a suitable option for large-scale production. However, tubular PBRs suffer from high power consumption due to the long pumping distances and high head loss arising from the high surface-to-volume ratio [115], regular wall cleaning maintenance [114], and sometimes photo-limitation, which can occur in outdoor cultivation [86] with larger-diameter tubes. Due to the length and relatively low flow velocities in the range of 0.20 $m \cdot s^{-1}$ to 0.50 $m \cdot s^{-1}$ [113], mixing is limited, and these systems can suffer mass-transfer problems [116]. In addition, the central part of the tubes may receive insufficient light, and hence growth is constrained [116]. However, these systems take benefit from plug-flow kinetics, which allow for high initial growth rates and the ability to easily stage nutrient availability, pH changes and illumination during culture development.

Compared to vertical tubular PBRs, horizontal tubular PBRs (HTPBRs) have a better angle for incident light, which aids in capturing maximum sunlight. However, this also

causes an increase in temperature, requiring expensive temperature-control systems [86]. HTPBR tubes are arranged in a horizontal fence-like structure, which raises the cost of operation [114]. HTPBRs are more practicable and scalable but are not economically feasible for large-scale production due to high land and cooling requirements [117].

Carlozzi and Sacchi [91] investigated an underwater tubular PBR to control the temperature of the reactor in an outdoor environment for PHA production using *Rhodopseudomonas palustris* 420 L. They found higher biomass productivity during the study period, with an average biomass yield of 0.7 g biomass dry weight·g^{-1} acetic acid. They found a PHA content of 4% of the dry biomass weight in summer and reported PHA up to 18% in unpublished data while concomitantly producing hydrogen. Likewise, in another study, Carlozzi et al. [45] investigated a particular type of tubular PBR, called L-shaped row PBR, for PHA production utilizing *Rhodopseudomonas sphaeroides* S16-VOGS3 under solar light by adopting a light/dark cycle. The novel PBR had five parallel rows with a total working volume of 70 L. Every row was an L-shaped loop with four pipes laid horizontally in demineralized water for temperature control, and eight pipes were placed one above the other and were connected with U-fittings, as shown in (Figure 6).

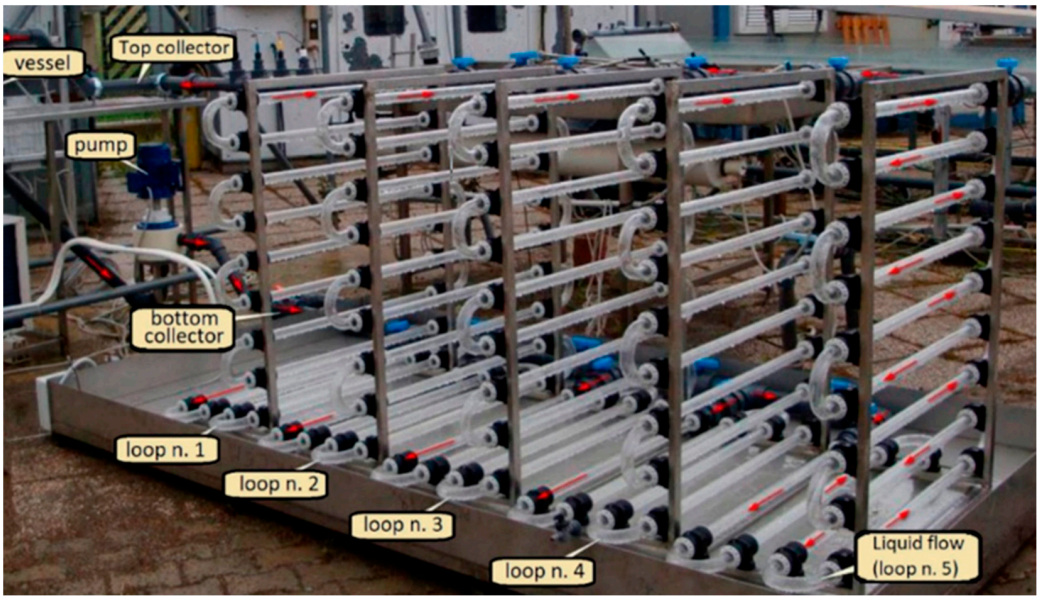


Figure 6. L-shaped water-cooled tubular PBR. Reproduced from Carlozzi et al. [45].

Using such a type of system was to avoid the usage of an external temperature-control system and maintain the culture temperature for maximum PHA-rich biomass. The study concluded that such a system design was feasible for optimal temperature control and solar-light capturing for producing PHA-rich biomass [45]. Such systems can be used for PNSB-PHA production without using expensive temperature-control systems.

Overall, tubular PBRs offer a unique solution for PNSB-PHA production by providing a controlled environment for PNSB metabolism. The closed, transparent tubes of tubular PBRs provide an anaerobic environment and allow for maximum light penetration, providing the energy source needed for photosynthesis and PNSB growth. The use of plug-flow kinetics in tubular PBRs can play a crucial role in maximizing PNSB growth and PHA production by allowing targeted nutrient delivery at specific stages along the carbon-removal and growth process, and ramped illumination as the biomass develops, which is a significant advantage of tubular PBRs. By optimizing the flow rate and mixing strategy, ideal energy and resource conditions for PNSB growth can be achieved.

PNSB's tendency to form biofilms can impact mixing requirements and pose challenges for efficient PNSB growth in tubular PBRs. Proper cleaning strategies must be employed to prevent the formation of biofilms, which can negatively impact PNSB growth and productivity. However, on the other hand the small size of PNSB cells and their tendency

not to aggregate strongly, except as surface biofilms, may negate the need for strong mixing requirements in the reactor. Another potential challenge in using tubular PBRs for PNSB growth is the production of hydrogen gas, which may build up in the reactor and can impact PHA production, requiring the use of hydrogen gas valves to prevent it from accumulating.

In conclusion, tubular PBRs offer a promising solution for PNSB growth and PHA production, but proper reactor design and operation, informed by a deep understanding of plug-flow kinetics, are crucial for maximizing PNSB growth and PHA productivity. The development of effective mixing strategies, the optimization of light penetration and nutrient delivery, and the management of hydrogen gas levels are key considerations for successful PNSB—PHA production in tubular PBRs.

5.1.3. Membrane Photobioreactor (MPBR)

An MPBR combines a typically cylindrical or flat-panel PBR with a membrane process (Figure 5c) for two primary purposes: firstly, to increase effluent quality, such as when integrating PHA production with wastewater treatment, and secondly, to increase the retention of biomass in the system, resulting in reduced biomass losses and higher biomass concentrations for harvesting. Different membrane processes (microfiltration, ultrafiltration, forward osmosis, gas exchange, ion exchange) and membrane configurations (flat sheet, hollow fiber) have been used in MPBRs [118]. A key benefit of MPBRs over other PBRs is their capacity to enable the independent control of SRT and HRT [119]. HRT is the fundamental working parameter of MPBR systems, as it directly decides the treatment capacity and nutrient loading of a bioreactor [120]. The virtually complete biomass retention of an MPBR allows shorter HRTs (lower than $2/\mu$ (typically from 6 h up)) compared to traditional PBRs, which generally have HRTs longer than 2 days [120]. This capacity not only produces high biomass efficiency (on account of the higher nutrient loading) but can also decrease capital costs [121].

The biomass productivity under steady conditions is approximately equal to the biomass wasting rate, which is inversely proportional to the SRT of an MPBR. Unlike membrane bioreactors (MBRs) that are normally operational at long SRTs (>15 d), a sensibly low SRT (around 10 d) was discovered to be useful for MPBRs in terms of accomplishing a higher biomass yield [120]. SRT additionally impacts the microbial community and the physicochemical properties related to auto-flocculation and the settling capacity of the biomass [122]. Thus, the viable control of SRT may prompt a more dynamic and harvestable MPBR culture. For instance, Luo et al. [94], while assessing performance parameters and operating conditions of an MPBR with the microalgae *Chlorella vulgaris*, discovered that shorter SRTs and HRTs were related to faster-growing, more suitable, and more homogenous cultures, while increased SRTs and HRTs advanced harvesting potential and nutrient removal. A study by Alloul et al., in a semi-continuous constantly illuminated PBR [123], found that different PNSB species dominated under different SRTs. They found that the optimal SRT for protein productivity of 0.64 g protein·L^{-1}·d^{-1} was around 0.19 days. A similarly shorter SRT could potentially benefit PHA production since a longer SRT will promote endogenous decay, including on stored PHA.

A lower SRT and the controlled dilution of biomass suspension could reduce the self-shading impacts of higher biomass concentrations and subsequently improve biomass production [94]. However, maintaining an MPBR at low SRTs requires a large waste sludge flow, which must be either retreated or discharged unfiltered. For instance, at a 2.5-day SRT, almost 40% of the bacterial culture from the MPBR would need to be thickened in extra downstream treatment [124]. To overcome the issue of harvesting, Parakh et al. [125] integrated a gravity settler with MPBR for in-situ biomass concentration and the harvesting of *Graesiella emersonii* (algae). The authors reported that their novel MPBR-settler system resulted in a concentrated biomass of 31.1 g·L^{-1}, which is significantly higher compared to traditional MPBR systems that only produced 0.2–3.4 g·L^{-1} of biomass. The authors concluded that the MPBR-settler system is a novel approach that allows operation at

low sludge-retention times (SRT) of 6–8 days, without causing a significant outflow of unfiltered effluent.

The MPBR also has various disadvantages, the primary one being membrane fouling. Membrane fouling accumulates microorganisms and organic and inorganic chemicals on the membrane surface and inside the membrane-permeable structure [126]. Membrane fouling is known to be mainly brought about by metabolites (i.e., EPS and soluble microbial products (SMP)) released or produced by microorganisms and cell materials, which depend on the specific microbial species and operational conditions. For instance, changes in environmental stress (i.e., salinity concentration) and operational conditions (i.e., temperature and DO) or rapid changes in microbial community and metabolism (compositions and production rates of EPS and SMP) may influence membrane fouling [127]. Membrane fouling is the main issue in algae-based MPBRs [128]. However, membrane fouling in the case of PNSB may be less of an issue due to PNSB's low EPS production [129]. However, the reduced shear flow associated with the anaerobic operation of the MBR could lead to increased fouling compared to more traditional (non-photo-based) aerobic MBR systems [126].

Chitapornpan et al. [95] conducted a study for treating food industry wastewater in an anaerobic MPBR. A glass vessel with an 8 L working volume was used as the reactor with IR illumination. During polymerase chain reaction–denaturing gradient gel electrophoresis analysis, 11 out of 56 bands were identified as PNSB belonging to *Rhodospirillum*, *Rhodoplanes* and *Rhodopseudomonas*. The PNSB community was resilient to fluctuations in the influent concentration of the real wastewater, with the biomass showing a net yield of 0.6 g-DS/g-BOD with 37–41% protein content.

Overall, in designing an effective MPBR for PNSB-PHA production, several key factors must be taken into account. Firstly, light penetration is crucial for PNSB growth, and the membrane layout should be selected to allow adequate light penetration through the PBR. Secondly, controlling the biomass concentration and SRT can make harvesting easier, and the PBR should be designed to regulate this by adjusting the flow rate and mixing conditions, without significantly compromising the growth rate. The choice of membrane material should also be based on its compatibility with the small size of PNSB flocs and resistance to fouling. While this system provides excellent effluent quality and retention of the fine PNSB biomass, its general geometry and advantages of high biomass concentrations provide some mismatch with PNSB-PHA production.

5.2. Immobilized Carrier Systems

Cell immobilization is the physical entrapment of bacterial cells to a certain defined region of space (carrier) that limits their free migration and produces hydrodynamic characteristics different from those of the surrounding environment. Benefits of immobilized systems include (i) provision of a regulating barrier to the bacteria from the environmental changes and conditions such as solvents, heavy metals, pH, and temperature; (ii) limited loss of bacteria in the effluent, reducing additional treatment [98] and maintaining high cell recovery; (iii) straightforward operation in a continuous flow; and (iv) ease in reusing cells [130]. This last benefit is of little significance for PHA production, as the extraction procedures to remove PHAs from the cells are destructive.

In general, cell immobilization can be roughly classified into three main categories: artificial (entrapped microorganisms), microbial aggregates and granular sludge, and moving-bed biofilm PBRs [96,102,106]. The advantages and disadvantages of each system are briefly described in Table 5.

Table 5. Advantages and disadvantages of immobilized-based PBRs systems.

Immobilized PBRs System	Advantages	Relevance to PNSB-PHA Production	Disadvantages	Relevance to PNSB-PHA Production
Artificial immobilization on or within suspended carriers	1. High surface area for microbial attachment and biofilm formation. 2. Improved mixing and mass transfer and improved control over nutrients. 3. Scalable design. 4. Reduced risk of contamination. 5. Carriers are frequently transparent or translucent.	1. Applicable, but with challenges for biomass harvesting and PHA extraction. 2. Applicable, nutrient transfer and nutrient control are important. 3. Applicable, but with challenges for biomass harvesting and PHA extraction. 4. Not directly relevant but can be helpful to avoid contamination of other bacteria. 5. Applicable, increases light capture.	1. Need for carrier material. 2. Higher energy requirements for mixing and aeration. 3. Potential loss of carriers during harvesting. 4. Reduced volumetric productivity compared to other PBRs.	1. Applicable, results in extra cost, particularly as they have to be recolonized after each harvest. 2. Applicable, results in high operational costs and energy consumption. 3. Applicable, reduced biomass and PHA production. 4. Applicable, less PHA production per unit volume.
Self-immobilized granular systems	1. High biomass retention and stability. 2. Higher volumetric productivity. 3. Reduced risk of contamination. 4. Lower energy requirements for mixing and aeration. 5. Ability to withstand shocks and fluctuating operating conditions.	1. Applicable, but more research is needed on PNSB stability in the system. 2. Applicable, increased PHA production in a smaller volume of the PBR system. 3. Applicable, prevents contamination from other microorganisms, which can affect PHA production. 4. Partly applicable, may be offset by higher illumination requirements due to light scattering from large aggregates at high concentration. 5. Applicable, improves the stability of the system and gives a broader range of application	1. Limited scalability. 2. Limited control of nutrient and O_2/CO_2 distribution. 3. Potential loss of biomass during harvesting.	1. Applicable, may pose a challenge for cost-effective PNSB PHA production. 2. PHA is stored under nutrient-limited conditions, for PNSB, O_2/CO_2 is not required. 3. Applicable, reduces the yield of PNSB-PHA production.
MBBPR	1. Effective mass transfer and good mixing. 2. High pollutant removal. 3. Less space requirements. 4. Higher biomass concentration. 5. Low HRT.	1. Applicable, enhances the supply of nutrients and light exposure. 2. Applicable, high pollutant removal results in high biomass or high PHA production. 3. Applicable, desirable. 4. Applicable and not applicable, higher PHA production rates and light scattering, respectively. 5. Somewhat applicable, balance required between loading, removal and biomass concentration.	1. Biofilm detachment. 2. Carrier materials need relocation during reactor maintenance. 3. Light attenuation.	1. Applicable, reduced biomass and PHA production. 2. Applicable, labor-intensive and time-consuming. May require relocation during PHA extraction. 3. Applicable, reduced biomass concentration and lower PHA productivity.

5.2.1. Artificial Immobilization on or within Suspended Carriers

An artificial immobilization system includes gel entrapments and encapsulation, covalent attachment, ionic adsorption on a thin layer, and water-insoluble matrices [98]. To date, various media have been examined for artificially immobilizing purple bacteria, including agarose, agar, alginate gel, nanoporous latex coating, polyurethane foam, polyvinyl alcohol boric acid gel, porous glass [98,131], cinder beads, and coconut fiber [132]. Since the 1980s, researchers have conducted various studies to produce hydrogen by adopting artificial carrier immobilized systems in which the carriers remain in the system [98].

Artificial immobilization on or within suspended carriers offers several advantages for PNSB-PHA production. The high surface area for microbial attachment and biofilm formation, improved mixing and mass transfer, and better control over nutrients can enhance the efficiency of the process. The scalability of this system and the reduced risk of contamination further add to its benefits [133]. However, there are also challenges associated with this system. The need for a carrier material [134] can add to the operational costs. The higher energy requirements for mixing and aeration can lead to increased operational costs and energy consumption [135]. There is also a potential risk of losing carriers during harvesting, which can result in reduced biomass and PHA production. These factors need to be considered when using this system for PNSB-PHA production (Table 5).

5.2.2. Self-Immobilized Granular Systems

Granular-sludge immobilization involves a self-immobilization community of bacteria [136]. Biological granules are self-supporting biofilms with a diameter of 0.2–2 mm and a layered microbial structure. Granular technologies are designed for improved final-sludge dewaterability, energy efficiency, and low footprint through high biomass retention and the integration of solids separation into the bioreactor vessel [137]. The advancement of granular systems has recently expanded towards resource recovery, with potential products including biofuels, fine chemicals, and biopolymers being produced from aerobic, anaerobic, and photosynthetic technologies [138,139].

In anaerobic systems, a suitable up-flow velocity (0.5–10 $m \cdot h^{-1}$) is a typical selection pressure [140] promoting a balance of biofilm growth rate and liquid shear flow, which ultimately results in a denser biofilm with a smooth surface [141]. These are typically achieved using effluent recirculation and taller (high height/diameter ratio) reactors [142], such as UASB reactors [96,143] and expanded granular-sludge-bed (EGSB) reactors [144]. An alternative approach is using settling selection pressure for granule development, which utilizes a sequencing-batch-reactor (SBR)-type process [145].

Sawayama et al. [96] studied the potential of a lighted UASB (LUASB) reactor for treating wastewater and producing PHB. The study found that the LUASB method is a promising option for both treating wastewater and producing phototrophic bacteria and PHB (15.1–25.3%) from various waste streams. The subsequent study by the same group investigated the performance of the LUASB reactor for wastewater treatment and PHB production under anaerobic–light and sulfate-rich conditions. The study found that the average PHB content based on dry bacterial biomass was significantly reduced to 1.4–3.6% [143], possibly due to an altered microbial community associated with sulfate reduction. Furthermore, the higher sulfate concentration may have resulted in a greater demand for reducing power, which may have decreased the carbon flux toward PHB synthesis. Overall, the difference in PHB content between the two studies highlights the importance of optimizing reactor conditions and substrate selection to maximize PHB production, but it also demonstrates that the LUASB method is a promising option for treating wastewater with phototrophic bacteria and simultaneously producing PHB.

More recently, Cerruti et al. [145] investigated the potential of an anaerobic sequencing-batch PBR for the enrichment and production of a concentrated, fast-settling, mixed culture of PNSB with high nutrient-removal capacity. The study found that the settling ability of the PNSB biomass increased over the study, with a settled biomass fraction rising from 12% to 97%. This was due to the formation of bioaggregates with good settling properties,

which led to a more efficient separation of the PNSB biomass from the treated bulk liquid, facilitating downstream processing for the recovery and valorization of the PNSB biomass rich in nutrients for the production of bioproducts such as PHA.

Stegman et al. [146] investigated the growth and formation of granular PNSB in laboratory-scale up-flow anaerobic column reactors. The study found that PNSB can form granular biomass within 50 days using up-flow velocities as the major driving force for granulation. The low up-flow-derived granular biomass had better settling properties than the high up-flow-derived granular biomass. Blansaer et al. [147] investigated the potential of an anaerobic up-flow PBR for the aggregation of *Rhodobacter capsulatus*. The study found that the optimal organic loading rate for aggregation was 6.1 g $COD \cdot L^{-1} \cdot d^{-1}$, resulting in a high sedimentation flux of 5.9 kg $TSS \cdot m^{-2} \cdot h^{-1}$. The study also found that the hydraulic retention time, COD-to-nitrogen ratio, and the nitrogen source can impact aggregation, emphasizing the importance of pre-treatment or application to suitable wastewater.

Together, these studies demonstrate the potential of various self-immobilized granular-system configurations for the enrichment and production of phototrophic bacteria and PHB from different waste streams, as well as the importance of factors such as illumination, nutrient availability, and hydraulic conditions for achieving high biomass production and aggregation. By understanding the factors that promote the growth and aggregation of phototrophic bacteria, these systems can be optimized for the cost-effective recovery of PHA from PNSB biomass.

Overall, self-immobilized granular systems provide high biomass retention and stability, which can lead to higher volumetric productivity. The reduced risk of contamination is another advantage of this system [148]. These factors can potentially enhance the production of PHA using PNSB. However, this system has limited control of nutrient distribution [149], which may pose a challenge to cost-effective PNSB-PHA production. The scalability of this system is also limited [149], which may restrict PNSB-PHA production on a larger scale. Although there is a low risk, there is the potential of losing biomass during harvesting [149], which can reduce the yield of PNSB-PHA production (Table 5).

5.2.3. Moving-Bed Biofilm Photobioreactor (MBBPR)

The fundamental idea behind the MBBPR system design is to have a continuously working biofilm reactor with small head loss, low clogging risk, and a high specific surface area of a biofilm. The high specific area for biofilm growth is accomplished by introducing small neutral-density carrier media that can flow along with the water by mechanical means in anaerobic reactors and aeration in aerobic reactors (Figure 5d). Filling carrier elements in the reactor volume vary from case to case, based on the required effluent standard. However, the maximum filling of 70% (by volume) is recommended to keep adequate carrier movement in suspension [150]. For PBR systems, a much lower carrier filling is optimal, to avoid light attenuation issues. MBBPR has several advantages over other biofilm systems, including good effective mass transfer, good mixing, high pollutant removal, and lower space requirements [99]. Compared to suspended-growth systems, MBBPR results in higher biomass concentration, lower HRT, no sludge recirculation, longer sludge age, and no sludge bulking issues [151].

MBBPR systems also have a few disadvantages, such as the detachment of biofilms from the carrier material and the carrier material requiring relocation during reactor maintenance [150]. Thus, to make MBBPRs successful for wastewater treatment, the characteristics and nature of carriers play a vital role. To date, different carrier media have been introduced into the MBBPR process, including a Kaldnes biofilm chip, polycaprolactone, high-density polyethylene [99], polyurethane sponge, polyvinyl alcohol gel, granular activated carbon, biodegradable polymer, nonwoven media, polyethylene plastics, and polymer foam pads [151]. Huerta et al. [152] reported that moving-bed biofilm carrier (MBBC) properties, including density, shape, internal and external walls, area-to-volume ratio, diameter, height, and filling ratio, can affect MBBPR performance. They added that carriers used in MBBPRs must have a density close to or less than water so that they can float easily. Carriers

with a cylindrical shape offer several advantages compared to those with complex shapes. Cylindrical carriers are better due to their reduced mechanical losses and improved hydrodynamic behavior, which results from fewer collisions among the carriers. Additionally, a short cylindrical shape allows light to penetrate more easily into the interior of the carrier, where it can reach the microorganisms growing inside. In contrast, carriers with complex shapes may hinder light penetration due to their irregular surfaces, which could potentially reduce their effectiveness in the MBBPR system. Once the shape has been considered, it is important to examine the role of internal and external walls of the carriers. Internal walls provide an increased surface area, promoting biofilm growth, while the external walls can be designed help to minimize friction between adjacent carriers, thus preserving the outer biofilm layer [152]. Regardless of the shape of the carrier, these aspects play a crucial role in the performance of the MBBPR system.

For PNSB-PHA production, it is important to consider the ease of harvesting the biofilm from the carrier. The carrier should be designed to facilitate the harvesting process, with compatibility for easy biofilm removal. Different pre-treatment methods such as those involving heat or alkalis can be used for harvesting biomass, but these methods can result in increased costs. The shape of the carrier is crucial in determining the ease of harvesting. An ideal carrier should be able to float easily in the reactor, allow for sufficient light penetration for biomass growth, and be able to be harvested without the need for expensive treatments.

While non-photo moving-bed biofilm reactors are well established commercially with over 1200 installations worldwide [153], MBBPRs are an emerging technology in the field of wastewater treatment. Only a few studies have considered photosynthetic systems [100], but no studies involve PNSB. For instance, a recent study investigated the cultivation of cyanobacterium Nostoc species BB92.2 using MBBPRs with non-transparent high-density polyethylene carriers. Researchers utilized two glass MBBPRs, one with a 1.5 L working volume for laboratory-scale and the other with a 65 L working volume for pilot-scale testing. Both systems employed the airlift principle for carrier circulation. To remove biomass from carriers, they were washed with 50% sulfuric acid for 1 h at 150 rpm and 25 °C. The researchers found that higher carrier concentrations may cause growth-limiting shading effects, but immobilization increased from 0.7 to 0.95 with increasing carrier concentration in the laboratory-scale system due to changes in the flow regime. They concluded that biofilms on the carriers were not suitable for biomass harvesting but could be used as catalysts in continuous processes, such as filtering nutrients or pollutants from wastewater without washing out the cells.

The authors of this review have trialed anaerobic benchtop illuminated MBBPRs with K1, K2, and K3 biofilm carriers but did not succeed with PNSB colonization. It is suspected that the lower EPS production of PNSB [129] may limit adherence to the carriers, although the same cultures have successfully adhered to other polymeric materials [154]. Woven-polymer shade cloth has been the most successful of the materials trialed for biofilm formation, which may have potential as carrier materials [155]. There remains a significant gap in the area of carrier design and adhesion for PNSB to successfully implement an MBBPR. However, the lighting setup and geometry of the PNSB-PHA MBBPR can be adopted similarly to the microalgae studies with different mechanisms of mixing, such as mechanical agitation. This approach eliminates the need for aeration, which is unnecessary for PNSB, and reduces the overall cost.

Overall, the MBBPR system ensures effective mass transfer and good mixing [99], which can enhance the supply of nutrients and light exposure for PNSB-PHA production. The high pollutant removal [99] can result in high biomass or high PHA production. The system also requires less space and allows for higher biomass concentration [99]. However, this system may face issues like biofilm detachment, which can reduce biomass and PHA production. The carrier materials may need relocation during reactor maintenance [150], which can be labor-intensive and time-consuming. Light attenuation can also reduce biomass concentration and lower PHA productivity. These factors need to be considered when using MBBPRs for PNSB-PHA production (Table 5).

5.3. Fixed-Growth Biofilm Systems

Biofilms are 3D colonies of microorganisms that attach to biotic and abiotic surfaces. The formation of biofilms involves four steps: initial attachment, establishment, maturation, and detachment [156].

Biofilm systems involve bacteria adhering to a solid surface or suspended material. They are superior to artificial systems (e.g., gel entrapment) due to higher substrate-conversion efficiency and better retention of active microbial mass [130]. Likewise, biofilm-based PBRs have advantages over suspended PBRs. Biofilm reduces the reactor size and medium volume needed to cultivate the same quantity of biomass [157]. High biofilm-biomass density enables energy-efficient harvesting and reduces dewatering needs. Biofilm-based PBR allows for efficient biomass harvesting with a mechanical system. Expensive concentrating equipment like centrifuges, filters, and tanks can be omitted, saving on operational and capital costs, as the dry matter content of the product is typically >15% [158]. The suspended fraction can be reused or exits with effluent if not recovered [159]. PNSB biofilm PBRs are expected to have costs similar to algal setups but may be more cost-effective since carbon dioxide supply is not required, no product (O_2) inhibition exists, and the systems can treat primary rather than tertiary wastewater [160]. Disadvantages in biofilm-based PBRs include the formation of gradients associated with pH, nutrients, and light over the biofilm [161]. Therefore, understanding the development and composition of biofilm is vital for the design and operation of biofilm-based PBRs [101]. Fixed biofilm systems include photo-rotating biological contactors (PRBCs), porous-substrate photobioreactors (PSBRs), and grooved-surface reactors (GSPBRs). The merits and demerits of these systems are briefly described in Table 6.

5.3.1. Photo-Rotating Biological Contactor (PRBC)

A PRBC consists of numerous closely spaced discs or a drum with lightweight, packed supports on a horizontal shaft, partially or completely submerged in wastewater (Figure 5e). The shaft rotates constantly by mechanical or compressed air drive, and a biofilm forms over the media's whole surface zone [162]. Vertical discs have a lower ratio between the footprint and cultivation surface than horizontal systems.

Rotation in a PRBC facilitates contact with the growth medium and gas-biofilm mass transfer, involving gases such as oxygen and carbon dioxide. PNSB prefers anaerobic photoheterotrophy but can tolerate micro-aerobic conditions [108,163]. Oxygen at the interface likely creates an aerobic skin layer on the biofilm, with PNSB beneath it. Light intensity per disk can be adjusted by altering disk diameters and distance. However, rotation speed affects biofilm performance: high speed increases shear flow and mass transfer, while slow speed reduces mass transfer, leading to the drying of biomass. Additionally, CO_2 and light are spatially separated from nutrients, leading to nutrient scarcity [161]. This separation does not hinder PNSB but can stimulate PHA production.

Wang et al. [101] effectively used a PRBC to cultivate anoxygenic-photosynthetic-bacteria (APB) biofilm, removing color and COD from azo dye wastewater, and identifying three APB species. In another study, they [164] found a single PRBC's biofilm to be more efficient than RBC's for removing nitrogen, phosphorous, sulfur, and carbon. No reports exist on PNSB-PHA production via PRBC, which would require proper lighting and complete anaerobic conditions.

Table 6. Advantages and disadvantages of fixed-biofilm PBRs.

Biofilm System	Advantages	Relevance to PNSB-PHA Production	Disadvantages	Relevance to PNSB-PHA Production
PRBC	1. High surface to footprint ratio. 2. Good contact with growth medium 3. Possibility of regulating light intensity 4. Efficient gas-biofilm mass transfer.	1. Applicable, increases productivity. 2. Applicable, efficient substrate and nutrient delivery 3. Applicable, light exposure can be optimized 4. Not applicable, PNSB does not require oxygen.	1. Rotation speed can cause effect on biofilm performance 2. Spatial separation of CO_2	1. Applicable, decrease in biomass production and hence PHA. 2. Not applicable, PNSB does not require large quantities of CO_2 during photoheterotrophic growth.
PSBR	1. Small volume requirements. 2. Biomass with low water content 3. Low energy requirements for harvesting, cooling, aeration, and mixing 4. Easy harvesting 5. High photosynthetic efficiency	1. Applicable, reduces capital and operational costs. 2. Applicable, greatly reduces PHA extraction costs. 3. Applicable, reduces operational costs and potentially increases productivity. 4. Applicable, beneficial for PHA extraction 5. Applicable, increased biomass production and PHA content.	1. High energy requirements for pumping 2. Large water losses due to evaporation	1. Applicable, high operational cost and hence uneconomical PHA production. 2. Applicable, usually these systems would aim for integrated water treatment which may target to reuse water. Suitable if water is to only be disposed.
Grooved	1. High specific surface area 2. Enhanced convective mass transfer for substrate and metabolic products.	1. Applicable, better light efficiency for same reactor volume 2. Applicable, can improve nutrient delivery to the PNSB, which can result in increased biomass and PHA production.	1. Biomass harvesting 2. High manufacturing cost	1. Applicable, PHA recovery requires extraction which is challenging due to complex structure of the grooved surface 2. Applicable, limits their feasibility and practicality for PNSB PHA production, especially in large-scale applications.

5.3.2. Porous-Substrate Photobioreactor (PSBR)

PSBRs are a recently developed technology and have to date only been applied for microalgal cultivation [102]. PSBRs have a microporous substrate that supports biofilm growth and transports liquid media, separating the liquid media and biofilm (Figure 5f). This new technology eliminates water from the biofilm surface, with the biofilm directly exposed to the ambient gas. This principle was first used in 2003 to stabilize algal biofilms for ecological purposes, then applied as a PBR and called the attached-cultivation system or twin-layer system. PSBRs use a porous sheet-like material, which enables bacteria to attach and form a biofilm. The porous-substrate material separates cells from the culture medium, allowing water, nutrients, and gases to diffuse and evaporate to the biofilm surface. PSBRs were designed to supply CO_2 to traditional PBRs but may not be suitable for PNSB due to oxygen at the interface. It is likely within this system for an aerobic skin layer of aerobic heterotrophs to form with PNSB below, similar to PRBCs. Productivity is expected to be low as the PNSB will receive less light, but it will be in direct contact with the substrate.

Materials used for the functional barrier include plain printing paper and synthetic nonwoven/textile combinations. Parallel multi-sheet panels enhance light distribution by 4-10x compared to a single sheet, improving light use and bacterial growth [165].

PSBRs provide benefits like smaller operational volumes, easy harvesting, enhanced biomass productivity, and high photosynthetic efficiency. Further optimization is possible with a light-diffusion system for even light distribution in the PSBR [166]. Additionally, PSBR technology minimizes energy use by eliminating cooling and mixing and simplifying harvesting [165]. It only requires water pumping to the PSBR top for gravity-led movement through the system.

To date, these systems have only been set up as small pilot-scale reactors [167] and research-facility-scale PSBRs [168]. For instance, Do et al. [102] studied laboratory-scale twin-layer PSBRs for astaxanthin production using microalga *Haematococcus pluvialis*. Nutrients in PSBRs diffuse from the substrate to the biofilm surface without mixing. In thick biofilms, nutrients reach the surface when sufficiently supplied with fresh medium [160]. Nutrient transport is also aided by evaporation-driven liquid flux, which is less effective under low temperatures and light [169]. No studies in the literature report using a PSBR for PNSB-PHA production. The authors have undertaken their own initial assessment of PSBRs using a benchtop system in an open atmosphere with various substrates like agricultural shade cloth, coconut fiber mats, polyester fiber filters, and jute bag, but they observed minimal biofilm growth.

Overall, PSBR systems require a small volume, produce biomass with low water content, and do not require energy for harvesting, cooling, aeration, and mixing [166]. These factors can reduce capital and operational costs, greatly reduce PHA extraction costs, and potentially increase productivity. The system also allows for easy harvesting [165] and has high photosynthetic efficiency, which can increase biomass production and PHA content. However, the PSBR system has high energy requirements for pumping, which can increase operational costs and impact PHA production. Large water losses due to evaporation [170] could be a concern if water is not intended to be reused (Table 6). Hence, PSBRs are unlikely to be the optimal reactor configuration for PNSB-PHA production, but further studies are required.

5.3.3. Grooved-Surface PBR

Grooved-surface PBRs increase biofilm adhesion area with their intricate 3D internal structure, initially designed for algal biofuel production and later tested for PNSB hydrogen production [103].

Zhang's grooved PBR, made from polymethyl methacrylate (PMMA) with dimensions 100(H) × 50(L) × 20(W) mm^3, had 1 mm deep wide-etched grooves on one main wall (Figure 5g). They explored the impact of operational conditions on light-conversion efficiency and hydrogen production. Compared to a similar-sized flat-panel PBR, the grooved

PBR increased hydrogen production and light conversion efficiency by ~75%, showing significant potential.

Grooved-surface PBRs, compared to flat-panel ones, offer advantages like higher surface-to-volume ratio, less light attenuation in the biofilm zone (leading to high light-conversion efficiency), and improved mass transfer due to enhanced reactor-surface turbulence [103]. The same researchers used a PMMA-made alveolar-panel photobioreactor of size 350 m × 180 m × 50 mm for its transparency, chemical stability, and ease of incision. The PMMA wall was incised to create 10 mm wide deep grooves, offering a rough surface for PSB cells to grow into a biofilm. The study revealed that *Rhodopseudomonas palustris* biofilm on the alveolar panel photobioreactor's grooved surfaces had superior continuous H_2-production capability compared to other suspended-culture studies [171].

Overall, grooved systems provide a high specific surface area and enhanced convective mass transfer for substrate and metabolic products [103]. This can improve nutrient delivery to the PNSB, resulting in increased biomass and PHA production, and provide better light efficiency for the same reactor volume. However, the use of grooved-surface PBRs for PNSB-PHA production also presents certain challenges that may affect the efficiency of PHA production. One of these is the difficulty in harvesting biomass [172] for PHA extraction, as microorganisms are attached to the groove surface. This issue can result in lower PHA yields and reduced productivity. However, certain chemicals and treatments may be available that can be used to harvest the biomass easily [173]. Cost is a further disadvantage of this type of system, due to the additional work and material required to allow for the etching process. In summary, while the grooved-surface design of PBRs has potential advantages for PNSB-PHA production, effective harvesting procedures need to be developed to realize the benefits of this system (Table 6).

5.3.4. Other Biofilm Photobioreactors

Hülsen et al. [160] conducted a study to produce single-cell proteins using purple phototrophic bacteria in an internally illuminated biofilm PBR using continuous and batch modes. The PBR used comprised of a cylindrical, Perspex main body with a flanged conical bottom. For biofilm development, the reactor lid was flanged to the vessel containing 17 sealed, hollow Perspex tubes submerged in the PBR liquid. Tubes were illuminated with two flexible, dimmable IR LED strips placed against each other to irradiate the PBR from the inside out. For biomass harvesting, the PBR was equipped with an internal rubber-wiper system. They reported that the biofilm PBR removes moderate amounts of COD, N and P while generating a consistent PPB-dominated biofilm. The batch mode was more effective than the continuous mode for harvesting the biofilm, as the biofilm partly redissolved and disintegrated in the continuous mode, limiting the actual biomass recovery. However, a well-compacted and stable biofilm can be achieved by adjusting mixing and shear flow [160].

6. Summary and Future Directions

This review paper focuses on the design of photobioreactors for producing PHAs using anoxygenic photoheterotrophs. The paper explores various types of systems, including suspended PBRs, immobilized carrier systems, and fixed-biofilm growth systems, and evaluates their potential for PHA production. The granular-sludge systems for the self-immobilization of bacteria are among the most promising of the various PBRs discussed in this review paper. These systems have demonstrated significant potential for producing PHA from PNSB biomass, eliminate the need for solid–liquid separation and improve dewatering, and have high biomass production. The use of granular systems for PHA production from waste streams is an area that requires further investigation, particularly with regard to optimizing factors such as illumination, nutrient availability, and hydraulic conditions. On the other hand, grooved-surface PBRs and porous-substrate PBRs have been rated the lowest among the PBRs discussed in this review paper. While grooved-surface PBRs offer advantages such as high surface-to-volume ratio and high light-conversion

efficiency, the challenges of biomass harvesting and the cost of reactor fabrication limit their current desirability and require the further optimization of their design. Likewise, porous-substrate PBRs offer advantages such as smaller reactor volumes for effective operation, easy harvesting, high biomass productivity, and high photosynthetic efficiency. However, for PNSB-PHA production under an open atmosphere, they are not successful, and they should be investigated under anaerobic conditions. In Table 7, different PBRs designs are evaluated for their suitability for PNSB-PHA production. Each PBR design is rated across multiple categories. These categories include:

Table 7. Comparison of different photobioreactor systems for PNSB-PHA production.

System	Illumination	Control of Nutrients	Mixing	Temperature Control	Biomass Production	Biomass Harvesting	Overall
Flat-panel PBR	4	4	4	4	4	4	**4/5**
Tubular PBR	4	4	4	4	4	3	**3.8/5**
Membrane PBR	4	4	4	4	4	3	**3.8/5**
Artificial immobilization on or within suspended carriers	3	4	4	4	4	2	**3.5/5**
Self-immobilized granular system	4	4	4	4	5	4	**4.2/5**
Moving-bed biofilm photobioreactor (MBBPR)	4	4	4	4	4	2	**3.6/5**
Photo-rotating biological contactor (PRBC)	3	4	4	4	3	3	**3.5/5**
Porous-substrate photobioreactor (PSBR)	4	4	4	2	3	3	**3.3/5**
Grooved-surface PBR	5	4	4	4	3	2	**3.3/5**

Rating scale: 1 = poor or very low, 2 = below average, 3 = average or acceptable, 4 = good or above average, and 5 = excellent or very high.

Illumination: This category measures how effectively the photobioreactor design allows for light penetration, which is crucial for the growth and metabolic activities of photoheterotrophic organisms.

Control of Nutrients: This assesses the PBR's ability to maintain optimal nutrient levels. This includes both the ability to replenish depleted nutrients and avoid the toxic build-up of excess nutrients.

Mixing: This category gauges the effectiveness of the PBR design in facilitating the homogenous mixing of cultures, thereby preventing gradient formation and ensuring the uniform exposure of organisms to light and nutrients.

Temperature Control: This category rates how well the PBR design can maintain an optimal temperature for the growth of PNSB, considering that extreme temperatures can inhibit growth or even kill the organisms.

Biomass Production: This category rates the potential of the PBR design to maximize the biomass yield, which is directly linked to the overall productivity of the system.

Biomass Harvesting: This assesses how well the PBR design accommodates the efficient and effective harvesting of biomass—an important aspect for the practicality and economic feasibility of the process.

Overall: This final category provides an overall rating, taking into account the average of all the aforementioned factors, to determine the most effective and efficient photobioreactor design for PHA production using PNSB.

Each of these categories is subjectively rated on a scale from 1 (very poor) to 5 (excellent), based on the available literature and empirical evidence. This rating provides a comparative overview of the different photobioreactor designs and their potential effectiveness in PNSB-PHA production.

Future research and development should focus on advancing the design and operation of PBRs to maximize PHA production using anoxygenic photoheterotrophs. General principles for improvement include enhancing biomass harvesting in fixed-growth sys-

tems, developing internal lighting systems like waveguides, and exploring system-specific variations. For instance, grooved PBRs could benefit from solvent-based methods, while MBBPRs may benefit from materials allowing for the straightforward chemical sloughing of biomass. An improved modeling of growth and PHAs production concerning light and carbon availability is also essential for better system evaluation. The potential of PRBC and PSBRs for PNSB-PHA production warrants further study.

Granular-sludge systems offer promise for PNSB-PHA production from various waste streams due to high biomass concentrations, ease of harvest and lower reactor costs. Future research could examine community dynamics and PHA production as influenced by factors like illumination intensity, shear flow, and substrate concentrations. This may include investigating the relationship between granule diameter and production efficiency.

In conclusion, continued research is necessary to optimize PBR design and operation to achieve cost-effective and scalable PHA production using anoxygenic photoheterotrophs, ultimately contributing to a more sustainable future.

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