Bioresource Technology 327 (2021) 124820

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

Up-scale challenges on biopolymer production from waste streams by Purple Phototrophic Bacteria mixed cultures: A critical review

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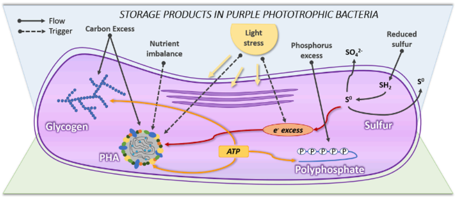
HIGHLIGHTS

- The production of biopolymers by purple phototrophic bacteria is reviewed.
- Understanding the underlying metabolic mechanisms for process optimization as a key.
- Special focus on polyhydroxyalkanoates and polyphosphate accumulation.
- Challenges for photobioreactors upscaling are critically defined.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Purple phototrophic bacteria
Polyhydroxyalkanoates
Polyphosphate
Photobioreactors
Anoxygenic photosynthesis

ABSTRACT

The increasing volume of waste streams require new biological technologies that can address pollution concerns while offering sustainable products. Purple phototrophic bacteria (PPB) are very versatile organisms that present a unique metabolism that allows them to adapt to a variety of environments, including the most complex waste streams. Their successful adaptation to such demanding conditions is partly the result of internal polymers accumulation which can be stored for electron/energy balance or as carbon and nutrients reserves for deprivation periods. Polyhydroxyalkanoates, glycogen, sulphur and polyphosphate are examples of polymers produced by PPB that can be economically explored due to their applications in the plastic, energy and fertilizers sectors. Their large-scale production implies the outdoor operation of PPB systems which brings new challenges, identified in this review. An overview of the current PPB polymer producing technologies and prospects for their future development is also provided.

1. Introduction

Worldwide, problems such as population growth, climate change and increased demand for food, energy and water, highlight the need for economic growth based on the principles of environmental sustainability. Environmental Biotechnology plays a key role allowing the optimization and recovery of resources and contributing to the improvement of both the overall benefit and the environmental quality of industrial processes (Dragone et al., 2020). Within this framework, biosynthesis of bacterial storage compounds offers new avenues for the development of tailor-made compounds suitable for industrial and medical applications. Although the synthesis of storage compounds consumes energy and nutrients, bacteria use them to persist and grow under a wide range of unfavorable and unstable conditions (Rehm, 2010). This is essential in environmental biotechnology applications since the use of heterogeneous organic wastes as feedstock is currently

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https://doi.org/10.1016/j.biortech.2021.124820
Received 27 November 2020; Received in revised form 29 January 2021; Accepted 30 January 2021

considered a necessity. Purple phototrophic bacteria (PPB) in mixed cultures constitute almost a paradigm of this kind of behavior.

PPB are probably the most metabolically diverse organisms found in nature. These organisms make use of bacteriochlorophyll pigments to obtain energy from wavelengths that are complementary to those used by oxygenic photosynthetic organisms, which express chlorophyll pigments. Indeed, while bacteriochlorophyll *a* and *b* present absorption peaks in the near-UV (<400 nm) and in the near infrared (NIR) light (800–1020 nm), chlorophylls only absorb in the visible (VIS) range (Scheer, 2006). From the sun irradiance that reaches the Earth surface, 42% occurs in the VIS range (400–700 nm) and 34% in the NIR range (700 – 1100 nm). Although a lower irradiance is available in the NIR region, PPB compensate this by presenting higher photosynthetic efficiencies in relation to microalgae that absorb in the VIS region (up to 8% in PPB and below 5% in microalgae) (Hülsen et al., 2014). PPB can use electrons from a wide variety of reduced molecules, both inorganics (photoautotrophy) as well as organics (photoheterotrophy). They also can grow without light by using organics as energy and carbon source through chemotrophy in aerobic and anoxic/anaerobic conditions (Capson-Tojo et al., 2020).

One of the most attractive features is their capability to thrive in illuminated anaerobic environments, where a high carbon recovery efficiency from waste streams can be attained with PPB due to the minimization of carbon dissipation through oxidative metabolism (Capson-Tojo et al., 2020). This allows the anaerobic operation of PPB systems with very low carbon footprint. The recent literature on the treatment of industrial and domestic wastewater (Lu et al., 2019; Puyol et al., 2019) and other urban and agricultural waste effluents (Allegue et al., 2020; Basak et al., 2014) demonstrates the unique metabolic versatility of PPB for recovering resources. Typical bioproducts to be sourced from PPB include hydrogen, single-cell proteins, fertilizers and other high added-value products such as polyhydroxyalkanoates (PHA), co-enzyme Q10, and 5-aminolevulinic acid (Capson-Tojo et al., 2020). However, scaling-up of the technology remains an untapped field, making difficult its industrial deployment.

Successful scale-up and economic feasibility of industrial scale production of biopolymers depends on various factors, such as the cost of precursor substrates, yield over substrate rate, volumetric productivity and the cost of downstream processing, among others (Rehm, 2010). While bioengineering aims at improving the upstream processes (use of low-cost substrates and increased productivity), bioprocess optimization of the upstream and downstream processes is required for scalable and cost-effective manufacture. In this review we analyze in detail the most promising storage compounds that PPB can produce as well as a critical analysis of the current state of the bioprocess scale-up and optimization.

2. Mechanisms for the accumulation storage compounds in PPB

Storage compounds are produced by living organisms and/or synthesized by processive enzymes that link building blocks to yield high molecular weight molecules. Depending on the conditions in which PPB are growing, four major classes of storage compounds are produced: zero-valence sulfur, glycogen, PHA and polyphosphate (poly-P). These compounds are stored intracellularly (and therefore are inclusions) except for sulfur which can be also stored extracellularly. The main environmental conditions for the accumulation of storage compounds as well as its industrial applications are schematically shown in Fig. 1.

2.1. Poly-P

Inorganic poly-P is the only polyanhydride found in all living cells forming linear polymers with variable chain length, which are constructed from repeating PO_4^{3-} and connected by high-energy anhydride bonds (Liang et al., 2010). It has been shown that poly-P affects numerous aspects of bacterial physiology, such as survival during the stationary growth phase, response to stress, motility, quorum sensing, biofilm formation or pathogenicity. The enzyme responsible for poly-P biosynthesis is the highly conserved poly-P kinase (PPK), which helps to store energy excess from light through phototrophic growth when low C / P ratios are present (Lai et al., 2017). Poly-P forms intracellular storage particles but may also form a membrane-anchored complex with low molecular weight polyhydroxybutyrate (PHB), facilitating the uptake of DNA and various ions (Reusch & Sadoff, 1988). This polymer can be accumulated as P reserve in response to periods of P starvation, but some organisms can accumulate Poly-P as energy storage (ATP), without requiring a previous P starvation phase. This capacity has been observed in phototrophs like microalgae (Solovchenko et al., 2016), PPB (Lai et al., 2017; Liang et al., 2010) and heterotrophs like the poly-P accumulating organisms (PAOs) (Desmidt et al., 2015). Some PPB species can accumulate internal PO_4^{3-} into the form of poly-P up to 13%–15% of its cell dry weight (Liang et al., 2010), nevertheless, there is still a great gap of knowledge regarding its accumulation patterns on PPB. Unlike poly-P accumulating chemoheterotrophic organisms, the driver for poly-P accumulation in PPB is related to the presence of high light availability, similarly to other photosynthetic microorganisms (Carvalho et al., 2019). Therefore, the process design is simplified as there is no need to have a succession of aerobic/anoxic stages in the photobioreactors, considerably decreasing the operation costs.

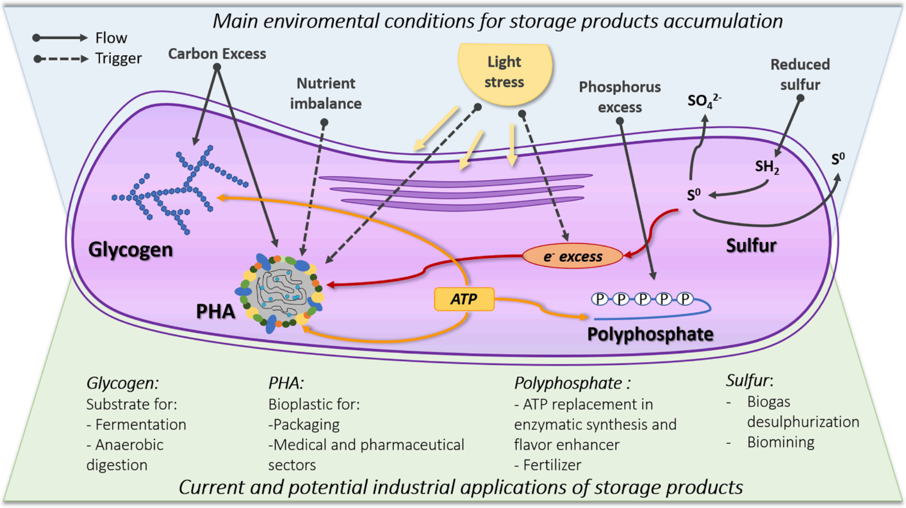

Fig. 1. Schematic representations of PPB anaerobic phototrophic metabolism that support biopolymers accumulation and their industrial applications.

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Phosphorus presents vast applications in the chemistry and food industry, but particularly, in the agriculture field where P is used as fertilizer (Solovchenko et al., 2016). Commercial production of bacterial poly-P for industrial applications is not currently economically feasible due to a much more efficient production by chemical synthesis (Iliescu et al., 2006). However, controlling the phosphorus propagation in nature is a highly important matter in environmental biotechnology, and the biological capture and recovery of P in waste is key for the protection of the aquatic environment. PPB systems are still under study for P recovery, which can boost the use of PPB biomass as C/N/P organic fertilizers (Sakarika et al., 2020).

2.2. Glycogen

Glycogen, a polysaccharide made up by glucose units, is another intracellular stored resource common in evolutionarily divergent species. It is a widespread form of carbon and energy storage that promotes survival during starvation (Sekar et al., 2020). Its role in bacteria is highly diverse. Glycogen contributes as an energy source when there is a lack of other energy sources, or as a temporary resource used during the physiological transitions required by dynamic environmental conditions. It can be accumulated in either stationary phase or under excess carbon and/or limited-growth conditions, and contributes to survival or maintenance in bacterial environments in which nutrient availability frequently fluctuates (Sekar et al., 2020). The glycogen biosynthesis capacity of PPB has been proved for a long time (Hara et al., 1973), and glycogen synthases (glgC and glgA) and hydrolase/transferase (glgX) were cloned from *Rhodobacter sphaeroides* (Igarashi & Meyer, 2000). However, there is still a gap of knowledge in the relation between PHA, and glycogen accumulation in PPB. A previous study indicated that *Rhodopseudomonas palustris* accumulate glycogen in higher percentage in almost all conditions (5–15% d.w.), synthesizing PHA when the environment has reducing equivalents in excess (0.3–7% d.w.) and the PPB are in static mode (De Philippis et al., 1992). However, glycogen accumulation remains almost constant, with decreasing values related to starvation conditions. The possible competition between glycogen and PHA accumulation in photoheterotrophic conditions, as well as the genetic regulation for this process, is still unclear. We may hypothesize that glycogen acts always as a carbon and energy storage during growth, whereas PHA regulates the electron balance, acting also as carbon storage during static mode. The energy required for glycogen is higher than that for PHB accumulation (starting from acetate, 1 mol of glycogen requires 4 ATP, 2 CO_2 and 4 reducing equivalents, whereas 1 mol PHB only requires 2 ATP and 1 reducing equivalent, (Fradinho et al., 2014)). However, glycogen is on the pathway of polysaccharides biosynthesis, and therefore it is the natural carbon storage process during growing conditions. PHA accumulation is more feasible in static mode, requiring less energy. Also, the lack of CO_2 is a trigger for PHA accumulation in detriment of glycogen, as Acetyl-CoA needs CO_2 for initializing any biosynthetic pathway (Bayon-Vicente et al., 2020).

The use of PPB-based glycogen as a carbon source for a variety of applications remains untapped. Recently, it has been studied the accumulation of glycogen in cyanobacteria to use the bacterial biomass as a feedstock for octanoic acid through dark fermentation (Comer et al., 2020), which may be a feasible alternative for PPB. In addition, the anaerobic digestion of PPB-based biomass has been recently studied (Hulsen et al., 2020). The role of glycogen in this process should be stated as it can potentially boost the biochemical methane potential of reject of PPB biomass coming from the extraction of higher value-added products. In this sense, glycogen may be a byproduct of biorefinery platforms. In any case, it interferes with most of the metabolic pathways of PPB and must be considered in any biopolymers' application of PPB.

2.3. PHa

PHA are linear polyesters, formed by the accumulation of carbon as reserve material in response to substrate excess when growth is limited owing to starvation of some nutrient, usually nitrogen, phosphorus or sulfur (Monroy & Buitron, 2020). PHA is deposited as spherical intracellular inclusion with an amorphous and hydrophobic PHA core that is mainly surrounded by proteins involved in such PHA metabolism (Jendrossek, 2009). The resulting PHA can be roughly divided into two groups, with short-chain-length (scl-PHA) of 3 to 5 carbons and with medium-chain-length (mcl-PHA) of 6 to 14 carbons. PHA is produced mainly through consecutive reduction of acetyl-CoA to (R)-3-Hydroxybutyryl-CoA, which is then reduced to PHA by PHA synthase (PhaC). PHA can be also produced by reduction of other Acyl-CoA, e.g. from the degradation or synthesis of fatty acids, or in other pathways involving reductive processes concurring to PhaC (Tsuge, 2002). PHA accumulation is enhanced in case of redox imbalance, to act as an electron sink (Bayon-Vicente et al., 2020; De Philippis et al., 1992), therefore sudden light increase could be a way to induce redox stress in the culture, thus forcing higher PHA accumulation. However, long-term cultivation could lead to metabolic adaptation and eventual decrease of PHA accumulation.

The most investigated PHA is the homopolymer poly-3-hydroxybutyrate (P3HB). Compared to the P3HB, PHA copolymers, composed of a mixture of hydroxybutyrate and hydroxyvalerate PHBV, show better mechanical properties with decreased stiffness and brittleness, increased flexibility and a decreased melting and glass transition temperatures, which allows for a wider temperature processing window and thus increase its processability (Albuquerque et al., 2011). A thorough review of PHB (homo- and copolymers) production with PPB mixed cultures has been recently published (Monroy & Buitron, 2020), where it has been shown the copolymer production capacity of PPB when using heterogeneous substrates (propionate among others), with up to 51% fraction of PHV moiety (Fradinho et al., 2014).

Currently, the production capacity for generating bioplastics through large-scale bacterial fermentation has reached ~ 2 M tons per year (Bioplastics, 2019). To date, industrial PHA production is carried out using pure microbial culture fermentation technology with high costs associated with carbon substrate (refined sugar substrates), fermentation operation and downstream processing. However, the relatively high cost (7–12 € PHA kg^{-1}) of PHAs compared to other biopolymers such as polylactic acid (2.5–3.0 € kg^{-1}), has hindered research activity into their use in commodity applications such as packaging and service items, and restricted their use to high-value applications, such as those in medical and pharmaceutical sectors (Koller et al., 2017). In any case, among bioplastics, PHA has the best biodegradability even in marine environments, but this feature has been barely considered when the production costs are calculated in cradle-to-grave approaches (e.g. life cycle assessment). An important aspect to consider in this context is the production by PPB of hydrogen as a by-product in the accumulation of PHA. This process have been widely studied in different bioreactors (Basak et al., 2014), and can be a possible strategy to improve the profitability of the overall process. A solar-powered photo-biorefinery could be as well a promising way for sustainable biodegradable PHA production, as will be discussed in subsequent sections.

2.4. Sulfur

PPB have the ability to grow using reduced sulfur compounds (mainly H_2S) as electron donors for their biosynthesis (Pokorna & Zabranska, 2015). Sulfide is oxidized to sulfate and, as an intermediate, elemental S^0 is produced and accumulated in the form of globules inside or outside the cells. In principle, S^0 can serve as both an electron acceptor (when it is being accumulated) and as a donor (Trüper, 1984), therefore sulfur compounds, and S^0 in particular, is expected to have a considerable influence on the production and/or consumption of H_2 by PPB (Laurinavichene et al., 2007). An excess of sulfur in the environment can induce sulfur accumulation of over 30% of the dry weight of the cell (Pedrós-Alió et al., 1985). Although there is no industrial scale of sulfur removal by PPB so far, this is a promising technology that can be used, for example, for biogas desulphurization. A previous study performed in the laboratory and in a pilot plant, achieved a complete oxidation of H_2S of the biogas using the purple sulfur bacterium *Ectofhiorhodospira shaposhnikovii* (Vainshtein et al., 1994). A recent paper have shown potential capacity of mixed cultures of PPB for photoautotrophic sulfide removal where the purple sulfur bacterium *Allochromatium* sp. predominated in the consortium (Egger et al., 2020). In this sense, mixed cultures of PPB may allow a potential technology for simultaneous wastewater treatment and biogas upgrading, including both biogas desulphurization, removal of carbon dioxide, and organic matter and nutrients removal (Marín et al., 2019).

Biomining is another option to use the ability of PPB to transform sulfide into sulfur globules. These organisms may be useful as intermediates in bio leaching of metals after metal recovery by sulfate-reducing microorganisms in a two-stage biological approach. Though this has been never tested with PPB, there are evidences indicating that sulfide-oxidizing microorganisms are suitable for these purposes (Suzuki, 2001).

3. Challenges in modeling the metabolism of PPB towards biopolymers production

Modelling is an important step in the synthesis, design, optimization, scale-up, and process engineering of biotechnological systems. Due to the extraordinarily complex metabolism of PPB, the quest for a comprehensive and usable model is challenging as it should be stoichiometrically correct (thereby should be based on metabolic mechanisms) and easy to be implemented in real plants. A comprehensive summary of PPB modeling can be found in Puyol et al. (2019). Most of the models published so far address the identification of metabolic mechanisms of PPB from a systems biology perspective (including energy, carbon, and redox flows), and are not suited to analyze the optimal process conditions to produce biopolymers without major simplifications. These models are also not useful as tools for decision-making in the biosynthesis of polymers through waste streams, since chemical, biochemical, and physical frameworks must be considered, as discussed in Batstone et al. (2019). Up to now, only two instances are found within this field, one dedicated to domestic wastewater treatment (Puyol et al., 2017), and another one focused on the description of the sulfur cycle in autotrophic conditions (Egger et al., 2020). However, both models lack integration with polymers production and therefore are only suited for specific applications.

Challenges for mechanistic modelling rely on the competition between electron and energy allocation mechanisms. Carbon competition between assimilation (to growth) and accumulation (as glycogen and PHA) has not been properly addressed in biochemical mechanistic models. The same happens with the competition for electron allocation between PHA accumulation, biohydrogen production and carbon dioxide fixation. The integration of these mechanisms needs also to consider the energy availability, where poly-P accumulation may play a key role that is still un-deciphered. In an aim towards simplification for obtaining a useful modelling tool, a potential way is the definition of artificial transient components that can serve as a link between energy, electrons and carbon allocation. We propose to define energy and electron pools as model components that can mediate between the different mechanisms. In any case, this needs thermodynamic integration, as well as a better understanding of photon-electron interaction that can serve as a basis to limit both the energy and electron availability. A complete analysis of the stoichiometry of genome-scale metabolic models based on key PPB species must set the boundaries for such an integration (Puyol et al., 2019). However, novel modelling approaches also must include the photon-electron interaction, including the efficiency of the transformation from light energy into chemical energy in the light harvesting complexes (LHC) and the reaction center that converts the quantum energy into chemical energy (Timpmann et al., 2014). This has been fully addressed for pure cultures and this information is particularly useful here (Timpmann et al., 2014). We propose to simplify the approach by defining a single model component that represents the concentration of light harvesting complexes that will drive the transformation of the quantum energy into chemical energy simplified in the energy pool. The production of LHC will be inversely related to the light intensity, as it is well known that high light intensity causes down-regulation of the transcription of BChl gens (Muzziotti et al., 2017). In addition, the presence of oxygen in oxidative conditions leads to bacteriochlorophyll degradation that deteriorates the phototrophic mechanisms (Zhu et al., 1986), and this must be also included in the model, thus predicting phototrophic inhibition in outdoor open systems. These new model components and how they interact with the different biochemical components are depicted in Fig. 2. The resulting model must be computed for conservation through energy and electrons, apart from chemical oxygen demand, C, N, P, S and ions that are mandatory for wastewater applications.

4. Potentials for PHA production as C recovery

As explained above, high production costs are the main limitation for PHA production at large scale. To decrease production costs, utilization of aerobic mixed microbial cultures (MMC) in non-sterile, open systems that can use low-cost, complex wastes as feedstock for PHA production (Reis et al., 2011), has been proposed. However, the search for low-cost processes that can sustainably produce biodegradable plastics is not restricted to aerobic systems and can be driven through PPB. In comparison to aerobic systems, PPB can also heterotrophically remove the organic carbon fraction from the waste streams but without requiring aeration (eliminating aeration costs). Moreover, a higher carbon conversion efficiency to biomass and PHA can be attained with PPB, minimizing substrate losses as CO_2 (Nakajima et al., 1997). As such, an increasing number of studies on the PHA producing capability of PPB have been reported in the last years, describing new strains, feedstocks and operating conditions that maximize PHA productivities. Thus far, the majority of the studies have focused on single strain processes (thoroughly reviewed by Capson-Tojo et al. (2020); Monroy and Buitron (2020)). Only few studies have explored the PHA potential of PPB mixed cultures, and in particular, the development of selection strategies to enrich them in PHA producing PPB (Fradinho et al., 2013a, 2016). An overview of PHA content and productivities reported for axenic and PPB mixed culture systems is provided in Table 1. Different operational conditions (batch, fed-batch, continuous operation) and carbon sources (organic acids, fermented wastes) have been evaluated on their suitability for PHA production with PPB systems. Thus far, the best polymer productivities were obtained with acetate as carbon source, with PPB mixed cultures presenting PHA productivities (0.62–2.11 g PHA L^{-1} d^{-1}) comparable with axenic cultures of *Rhodobacter sphaeroides* (1.22–1.57 g PHA L^{-1} d^{-1}) (Table 1). This likely results from the strong selection pressure imposed to mixed culture systems that favours the growth of high PHA storing bacteria. Regarding other carbon sources, some studies reported that real wastes like fermented palm oil mill effluent (Hassan et al., 1997) or fermented cheese whey (Fradinho et al., 2019) also suit to be used as feedstock for PHA production with PPB. Moreover, PPB cultures could successfully accumulate PHA under transient light (Fradinho et al. 2013b, with mixed culture) and under outdoor conditions (Carlozzi et al. 2018, with axenic culture), which combined with their demonstrated capacity to use real wastes as carbon source, forth comes the larger scale outdoor operation of PPB systems fed with waste streams. In this case, the operation of PPB mixed cultures is fundamental if complex wastes are used as feedstock. Although this is a recent research field, it is quickly developing, leveraged on the large knowledge acquired along the years on PHA production with single strains PPB, but also, with aerobic MMC.

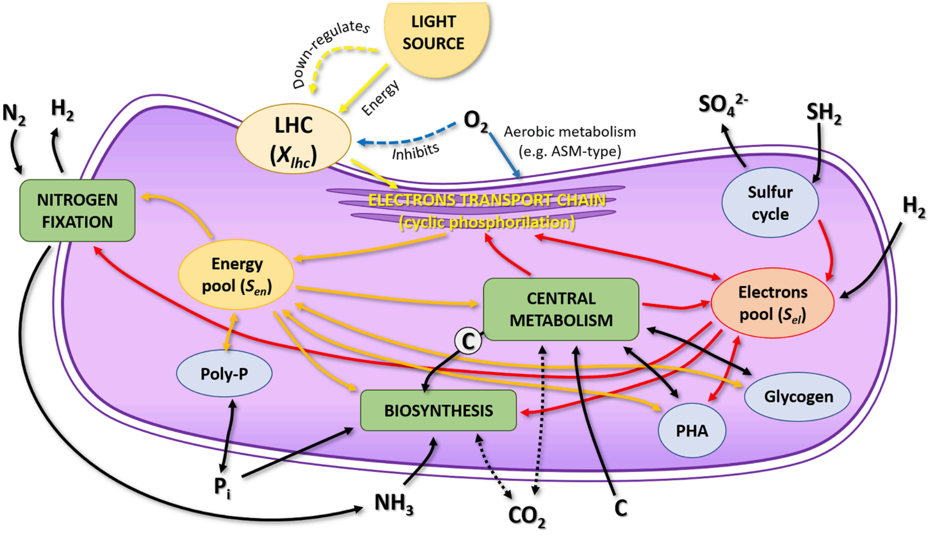

Fig. 2. Biochemical interactions in purple phototrophic bacteria as a basis for the development of a mechanistic kinetic model. Inputs and outputs from the model are represented in grey. Biopolymers are represented in blue. Key metabolic mechanisms are represented in green. New proposed transient components are the concentration of light harvesting complexes (X_{lhc}, golden), energy pool (S_{en}, yellow) and electrons pool (S_{el}, red). Color of the arrows represent main flows: quantum energy (light yellow), chemical energy (yellow), electrons (red) and nutrients (black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
PHA production by axenic and mixed PPB cultures.

Inoculum	Carbonsource	Reactor	Biomassconcentration	PHA content (%)	PHA productivity (g PHA L^{-1} d^{-1})	Productiontime	Selectionconditions	Reference
Rhodobacter sphaeroides IFO12203	Fermented palm oil mill effluent	Continuous	3.0–3.5g dcw L^{-1}	30%-60% dcw	0.048 – 0.072	–	n.a.	Hassan et al. (1997)
Rhodobacter sphaeroides RV	Acetate	Batch	2.2 g dcw L^{-1}	40% dcw	1.57	13 h	n.a.	Khatipov et al. (1998)
Rhodobacter sphaeroides U7	Acetate	Batch	2.7 g dcw L^{-1}	87% dcw	1.22	36 h	n.a.	Kemavongse et al. (2008)
Rhodopseudomonas sp. S16-VOGS3	Lactate	Outdoor, Fed-Batch	2.9 g dcw L^{-1}	20% dcw	0.063	144 h	n.a.	Carlozzi et al. (2018)
PPB enriched mixed culture	Acetate	SBR	1.9 g VSS L^{-1}	20% VSS	0.62	13 h	FF	Fradinho et al. (2013a)
PPB enriched mixed culture	Acetate	Transient Light, SBR	1.0 g VSS L^{-1}	30% VSS	1.39	4 h	FF	Fradinho et al. (2013b)
PPB enriched mixed culture	VFA mixture	SBR	3.0 g VSS L^{-1}	20% VSS	0.92	14 h	FF	Fradinho et al. (2014)
PPB enriched mixed culture	Acetate	SBR	1.8 g VSS L^{-1}	60% VSS	0.83	72 h	PF	Fradinho et al. (2016)
PPB enriched mixed culture	Acetate	SBR	1.6 g VSS L^{-1}	30% VSS	2.11	4 h	PF	Fradinho et al. (2019)
	Fermented cheese whey	SBR	1.4 g VSS L^{-1}	20% VSS	0.49	–	PF	

Dcw - dry cell weight; n.a. - non-applicable; FF – Feast and Famine; PF – Permanent Feast.

4.1. Enriching phototrophic mixed cultures in PHA accumulating PPB

In order to develop strategies that promote PHA production in PPB mixed cultures, it is important to recall that in PPB, PHA can be stored as a carbon reserve but also, as a sink for reducing power. Therefore, strategies can be design by specifically targeting each function. To this moment, studies were mostly conducted with synthetic feedstock and indoor artificial illumination, creating a knowledge base for higher complex system operation with real wastes in outdoor conditions.

4.1.1. Feast and famine (FF) strategy

To select for PPB that store PHA as carbon reserves, a Feast and Famine (FF) strategy can be applied. This is the strategy typically used in aerobic MMC and consists in applying transient carbon availability conditions, which continuously selects for organisms capable of storing PHA during a short carbon feast phase and consuming it during a long famine phase. Repeated FF cycles create a selection pressure that enriches the culture in organisms with high PHA storing capacity. Since this strategy requires the presence of an electron acceptor for the PHA consumption in the famine phase, Fradinho et al. (2013a) proposed the operation of mixed cultures comprised of PPB and microalgae, with the latest being the oxygen providers. When the consortium is operated in a FF regime, the system is not completely anaerobic due to the oxygen production by microalgae, but it is under sub-oxic conditions. During the Feast phase the ORP can drop to −300 mV and PPB use the photosynthetically generated ATP to anaerobically take up external carbon and store it as PHA (Fig. 3A). In this situation high carbon conversion efficiencies can be expected. During the famine phase, the cells oxygen demand decreases, and the oxygen that is continuously produced by microalgae, increases the ORP up to + 0/+50 mV. Oxygen values are never detected, minimising an eventual inhibition of PPB pigments expression.

Studies under these FF conditions and continuous illumination have shown the mixed cultures capability of accumulating up to 20% gPHA/gVSS, producing a PHA co-polymer (HB:HV molar fraction of 84:16) when fed with VFA mixtures (Fradinho et al., 2014). When the cultures

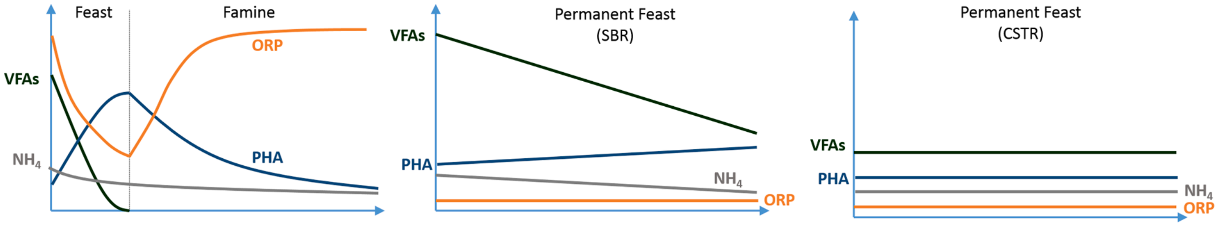

Fig. 3. General profile of phototrophic mixed cultures during PHA production under different selection strategies and reactor operation. A: Feast and famine selection in an SBR; B: Permanent feast selection in an SBR; C: Permanent feast selection in a CSTR. PHA: Polyhydroxyalkanoates, VFAs: Volatile Fatty Acids, ORP: Oxidation-Reduction Potential.

were operated under dark/light cycles, microalgae levels could be decreased relatively to PPB, further enriching the culture in PPB and enabling PHB contents up to 30% (Fradinho et al., 2013b) (Table 1). In this case, the PHB storage yield reached 0.90 ± 0.09 Cmol PHB/Cmol Substrate (acetate + internal glycogen) confirming that a high carbon recovery and conversion to PHA is possible during the feast phase.

Overall, studies under the FF strategy indicate that it is possible to efficiently enrich the mixed cultures in PHA accumulating PPB by promoting PHA storage as carbon reserves. The cultures not only can produce PHA co-polymers when fed with VFA mixtures (compounds commonly found in fermented organic wastes) but are also robust to transient illumination, an important feature for outdoor operation.

4.1.2. *Permanent feast (PF) strategy*

Another strategy that can be used to select for PHA accumulating PPB, explores the PHA storage as a sink for reduced power. Firstly, the PPB mixed culture must be cultured in an illuminated anaerobic environment under the permanent presence of VFA (so called permanent carbon feast, PF) (Fig. 3B and C). In these conditions PPB do not have access to the typical electron acceptors (oxygen, nitrate) and when they take up the organic carbon, PPB must activate metabolic pathways that allow electrons dissipation, thus balancing the cell internal redox state. PHA accumulation, CO$_2$ fixation via Calvin-Benson-Bassam (CBB) cycle and N$_2$ fixation/H$_2$ production via nitrogenase enzyme are some metabolic processes that can be used by PPB to dissipate electrons (Alsiyabi et al., 2019; McKinlay & Harwood, 2010). The key is to provide operating conditions that select for PPB that favour the PHA accumulation pathway. As such, if the PPB mixed culture is grown on feedstocks that contain NH$_4^+$ (which is usually the case in municipal wastewaters and some fermented organic wastes), nitrogenase expression is repressed and electrons cannot be dissipated through N$_2$ fixation/H$_2$ production (Koku et al., 2002). Regarding CO$_2$ fixation via CCB cycle, this is an important pathway used by PPB to achieve redox homeostasis, especially when the cells are growing on organic substrates more reduced than the biomass (Koku et al., 2002; McKinlay & Harwood, 2010). However, the CCB cycle is an ATP dependent and very high energy-demanding pathway (Alsiyabi et al., 2019). On the contrary, no ATP is required if reduced cofactors are re-oxidized via PHA production (Laycock et al., 2013). With both pathways at hand, the organisms that favour electron dissipation via PHA production have more energy available, which benefits their growth and enriches the PPB mixed culture in PHA storing bacteria.

It is important to point out that with this PF selection strategy, PHA production can occur side by side with cell growth, with no detriment to the culture selection (Fradinho et al., 2019, 2016). It is natural, however, that more PHA can be stored if the carbon precursors are not being used for growth. This can be achieved by limiting growth (nutrient limitation) and/or by increasing the light availability which allows cells to take up more VFA than necessary to grow. This was observed in a study by Fradinho et al. (2016) where a PPB mixed culture selected under permanent acetate feast, P limitation and low light availability could accumulate up to 60% PHB when exposed to higher light availability in accumulator reactors (Table 1). Also, high PHB storage yields of 0.83 ± 0.07 Cmol PHB/Cmol Acetate and global carbon yields (ΣY accounting biomass + PHB + glycogen production) of 1.03 ± 0.05 Cmol/Cmol Acetate could be achieved (Fradinho et al., 2019), further reinforcing the potential of PPB systems for high carbon recovery during PHA production.

The results show that targeting PHA storage as an electron sink is an interesting strategy that allows PPB mixed cultures enrichment in PHA storing PPB. In anaerobic and permanent presence of carbon (PF) the culture becomes fully enriched in PPB (algae are outcompeted) and high PHA content and carbon recovery can be achieved with light energy demands that can be supported with natural sunlight illumination (Fradinho et al., 2019).

5. Potentials for Poly-P production as P recovery

In the last decades, the world requirement for more pastures and crops for food production has increased the phosphorous demand, and since P is mostly obtained from finite phosphate rock mines, there are concerns that P rock reserves may become depleted (Solovchenko et al., 2016). Furthermore, phosphate widespread use and uncontrolled leakage into waterways can cause detrimental environmental problems, as eutrophication, with profound negative effects in the ecosystems (Solovchenko et al., 2016). Therefore, phosphorus recovery methods like the EBPR systems, have been implemented and widely used in wastewater treatment plants (WWTP) to recover phosphate from waste streams. In these systems, Poly-P storage requires intensive aeration (with associated costs) and part of the organic carbon removed from the wastewater is dissipated during PAOs' aerobic respiration. Implementation of photosynthetic processes can overcome these drawbacks, exploring for example the PPB capacity to store Poly-P, and by designing specific systems to anaerobically recover P from waste liquid streams. Studies with PPB strains from the species *Rhodobacter sphaeroides* (Hiraishi et al., 1991) and *Rhodopseudomonas palustris* (Lai et al., 2017; Liang et al., 2010) showed that under anaerobic illuminated conditions, and upon entering stationary growth phase, these organisms accumulated substantial amounts of Poly-P. Unlike the chemoheterotrophic bacteria in EBPR systems that obtain energy by consuming organic carbon sources, ATP production in PPB is independent of carbon uptake. Therefore, with ATP constantly available from light, PPB could continue to take up phosphate, and because growth was limited, cells were no longer assimilating phosphate into biomass, but instead, storing it as Poly-P. These findings are even more interesting considering that *R. sphaeroides* could accumulate 2–4% of their cell dry weight as phosphorous and *R. palustris* could achieved P contents of 4 – 10%. These values are in the range of P contents observed in PAOs, 5–15% (Liang et al., 2010) demonstrating the high storage potential of PPB.

In PPB mixed cultures, polyphosphate storage has also been reported, for instances, in cultures that were grown under P limiting conditions, in a system that aimed PHA production (Fradinho et al., 2019, 2016). In these studies, the mixed cultures were operated under low illumination conditions in the selector SBR and when exposed to high light availability, the phosphate uptake rates increased and up to 95% of the removed phosphate was accumulated and not assimilated into the biomass (Fradinho et al., 2016). Despite the culture's previous conditioning to P starvation periods, the higher light supply in this study demonstrates the importance of an excess of ATP availability to boost phosphate uptake and Poly-P storage.

The common finding between all these studies is that a surplus of ATP seems to be the key factor that triggers high P storage. As such, a PPB system for P removal from wastewater can be devised by including periods with excess of ATP availability. These conditions can be achieved by controlling several parameters like the light supply, which directly impacts ATP production, but also, by regulating the activity of some metabolic pathways that compete for ATP (e.g. ensure presence of ammonia to inhibit H_2 production). However, it cannot be excluded the hypothesis of combining poly-P storage with PHA production if the operating conditions allow an excess of ATP capable of sustaining both phosphate and high carbon uptake. Indeed, simultaneous Poly-P formation and PHA storage was observed in (Fradinho et al., 2019, 2016) and Lai et al. (2017) with mixed and single strains of PPB, respectively. The prospect of implementing such a PPB system to waste streams processing would be not only very economically attractive, but also, environmentally beneficial, due to the concurrent P and C recovery, and discharge of a better-quality effluent.

From the studies on P storage in PPB, Table 2 proposes a simple description of the different possibilities of producing Poly-P in combination (or not) with other compounds, assuming that cells have external P and ATP in excess for growth.

Mostly all the situations lead to Poly-P production, except for PPB growth on NH_4 limited conditions and presence of TCA organic acids, condition known for promoting H_2 production, and that competes with Poly-P for energy. A small note must be given regarding the Poly-P production with NH_4 presence and low carbon, because in this situation it is fundamental to filtrate the visible light to prevent microalgae growth under such nutrient rich environment. With this filtration assured, PPB systems also become a potential technology for wastewater treatment and P recovery from streams with low COD/P ratio.

6. Upscaling challenges and research needs

At the current moment, a large amount of knowledge has been harnessed on PPB technologies, and these are starting to be implemented in outdoor pilot/demonstration conditions for full evaluation of their potential. This technology transfer from lower laboratory scales to larger facilities is usually associated with upscaling challenges that must be identified and tackled.

6.1. Regional limitations and challenges

As a light dependent technology, large scale operation of PPB systems must consider the conditions of the implementation site in terms of local climate (sunlight availability) and land requirement (enough space to accommodate the facilities). Insolation is not uniform around the globe with some regions receiving higher irradiance levels than others and thus, being more adequate for the implementation of photosynthetic process. Also, due to the Earth's tilt, the day length changes along the year, with the variations between day/night time becoming more intense with increasing latitudes. These seasonal variations will strongly impact the system operation during the winter periods and imply process adjustments (e.g. OLR, SRT) if an all-year operation in planned.

Local temperature is also an important factor to consider when selecting the operating site. The very high temperatures generally found in regions with the highest insolation may be excessive for PPB activity that preferably grow in the range of 25 to 35 °C (Chen et al., 2020). On the other hand, Hülsen et al. (2016) reported that PPB cultures can adapt to lower operating temperatures (10°) with performances comparable to temperate temperatures (22 °C), which enlarges the regional applicability of PPB systems.

The implementation of PPB systems is very dependent on the space availability. Like in other photosynthetic processes, a high illuminated surface to volume ratio is fundamental to increase productivities. While different reactor configurations have been proposed to increase this ratio (e.g. tubular reactors, flat panels) their high investments and maintenance costs are prohibitive for large waste streams applications (Gupta et al., 2019). Open raceway ponds are a cheaper and simple alternative that can support the anaerobic growth of PPB mixed cultures, providing that organic carbon is present, which maintains the system under zero oxygen concentration levels. However, open ponds present liquid heights between 15 and 30 cm, thus requiring a high ground surface area in order to process high volume loads. As such, their implementation is suited for the outskirts of small towns or nearby waste producing industrial areas. This last case would be ideal for PPB systems since there would be (i) an easy access to the waste stream, (ii) access to qualified technicians for the system operation, (iii) possibility to integrate the PPB polymer production system with downstream and polymer processing units within the industrial zone, establishing a grid that follows the biorefinery concept. It is important to mention that the utilization of products recovered from waste streams is subject of national regulation. Therefore, an effective communication between technology developers, companies,and political institutions is required in order to develop standards that ensure the quality and safety of the PPB technology and of its products.

6.2. Bottlenecks on upscaling artificial light systems

Unlike sunlight that has daily/seasonal irradiation variations and is affected by weather conditions, artificial light can be fully controlled in terms of irradiation time, intensity, wavelength and spatial distribution. In the case of light wavelength, the utilization of LED lamps that emit in the near infrared region (NIR) can even be used to selectively grow purple bacteria over microalgae (Puyol et al., 2017). From a process point of view, artificial illumination would be the optimal light source for increased productivities of biomass and associated biopolymers (Fradinho et al., 2019). But, from the economical side, it brings extra capital and operating costs. Capson-Tojo et al. (2020) estimated an illumination cost of 1.9 $ per kg of PPB biomass grown under artificial LED illumination (based on 2019 US energy costs and assuming a maximum empirical value of 59 g COD/kWh for the biomass energy yield). In a biopolymer production process, which must also account with biomass downstream processing costs, these illumination costs would be a further weight that likely could not be compensated from the incomes of PPB biopolymers produced from waste streams. Similar observations have been made along the years in relation to microalgae bioprocesses in which the utilization of artificial illumination is only advised for high value molecules production (Blanken et al., 2013). As

Table 2
Proposed combinations of Poly-P production with other compounds by PPB under different C, N, S cultivation conditions, assuming external P and ATP in excess for growth. TCA: Tricarboxylic acid cycle.

	High Carbon		Low Carbon or Low S
	VFAs	Organic acids(from TCA)	
NH_4 present	Poly-P + PHA(Fradinho et al., 2016)	Poly-P(Hiraishi et al., 1991)	Poly-P(Hiraishi & Kitamura, 1985)
NH_4 limited	Poly-P + PHA(Lai et al., 2017)[1]	No Poly-P; likely H_2(Hiraishi & Kitamura, 1985)	Likely Poly-P(no references)

[1] glutamate was used as N source

such, free sunlight illumination is fundamental for the economic viability of PPB systems dedicated to the production of commodities, like PHA or fertilizers, which have lower commercial prices. Hence, PPB technologies should be designed in accordance to the constraints of solar irradiance and set-up for outdoor operation.

6.3. *Limitations of light irradiation based on rector design and potential measures to solve these limitations*

As stressed above, light irradiance is a key factor for the development of PPB bioprocess. These organisms can be cultivated via open or closed systems. Open raceways systems made of closed loop recirculation channels, are typically shallow (<0.3 m deep), unlined, and have a moderate surface-to-volume ratio of 3–10/m (Gupta et al., 2019). These systems are also prone to contamination by pathogens and predators, experience water loss via evaporation and have a low illumination efficiency. Nevertheless they account for 95% of the total worldwide algal production (Martin et al., 2013) due to the low capital and operation costs and easy scalability and operation. Closed systems or PBRs were designed to overcome the problems associated with open-pond systems, and culture growth in them is much more controlled. PBRs can be tilted at different angles using diffuse and reflected light that can play an important role in productivity. Although there are many examples of different PBR designs, two main types are commercially applied nowadays: tubular reactors, and flat panel reactors. Both have already been heavily studied on PPB (Lu et al., 2019), although mainly as indoor reactors. Regardless of the type of reactor used, sunlight is still the key limiting factor due to low light intensity, un-even distribution, shifts during day/night cycle, changing weather conditions, seasonal changes or direct exposure to UV irradiation. This light limitation does not allow to fully explore the growth potential of PPB and production of associated polymers. Fradinho et al. (2016) reported specific growth rates values around 0.5–0.8 d^{-1} in PHA producing PPB mixed cultures selected under low light conditions. However, specific growth rates can range from 0.6 up to 12 d^{-1} in axenic cultures (Madigan & Gest, 1979). This variation naturally results from the diversity of PPB organisms that was tested, but also, from the culturing conditions where temperature, carbon source and light irradiation needs are known to impact the growth rates. For comparison purposes, in PHA producing aerobic mixed cultures, specific growth rates have been reported in the range of 0.96 to 2.16 d^{-1} (Oliveira et al., 2017), but in these systems, culture selection occurs in the constant presence of oxygen (minimal oxygen saturation levels ~ 10%) supporting the culture's oxygen needs for ATP production and consequent growth. In photosynthetic systems, the difficulty in providing the ideal light input will limit ATP production and the capability to reach higher specific growth rates. This can explain why in comparison to aerobic chemotrophic organisms PPB systems will typically present lower biomass production rates. As such, it is fundamental to develop new strategies to enhance solar illumination efficiency, exploring different solar collection and distribution systems, thermochromic materials or solar filtration technologies.

6.3.1. *Solar collection and spatial light dilution systems*

Spatial light dilution is a method to decrease photon flux density and distribute it in a larger surface area. This method requires diffusors and collectors such as optical fibers, parabolic discs, green solar collectors or luminescent solar concentrator panels. Optical fibers are glass or plastic transparent fibers that can transport light from one place to another (for example, from a parabolic disc to inside the reactor). They have shown significantly increased productivities in microalgae cultivation and have already been studied on PPB, with 1.38 fold increase in hydrogen production (Chen et al., 2008). A breakthrough in new materials for a cost-effective production of optical fibers must occur since their excessive prices still makes them unsuitable for large scale application. An interesting option is the use of poly-methyl-methacrylate as a basis material. This material has been proved to be efficient in increasing the phototrophic growth of the green algae *Haematococcus pluvialis* in around 95% (Wondraczek et al., 2019). Interestingly, multi-wavelength light penetration through these optical fibers seems to be better in the near infrared range, so it is a promising material for being used with PPB. However, biofilm formation over the optical fiber is a major drawback for continuous operation if the process is focused on biopolymers production, which considerably limits the volumetric productivity. A potential solution for this is the application of smart low-voltage electric field to avoid bacterial adhesion over the illuminated surface. This has been successfully applied for avoiding problems of microbial fouling in MBR reactors (Zhang et al., 2015).

The most promising spatial dilution method are luminescent solar concentration panels, built by luminescent particles like organic dyes or quantum dots that absorb and re-emit light at longer wavelengths (Hill et al., 2019). The use of luminescent solar concentrations has already been tested on raceway reactors for microalgae and cyanobacteria cultivation with Raeisossadati et al. (2019) finding a 26% increase in biomass productivity for *Arthrospira platensis*. Luminescent solar concentrations are easy to construct, cost-effective and feasible to be used in outdoor open pond systems, but they must be tested on PPB, since the wavelengths needed to improve productivity are different from microalgae and cyanobacteria.

6.3.2. *Solar filtration technologies*

The development of wavelength filtration systems has allowed the specialized culture of PPB using only IR or NIR wavelengths. The more typically used method in laboratory scale PPB cultivation is UV–VIS absorbing foil, (Allegue et al., 2020), but even though it is a cheap material, its use in outdoors large-scale reactors does not seem suitable, since it is an easily tear-off material. Another option is colored filter glass, which is a relatively inexpensive type of filter, which attenuates light but sacrificing absorption. The design of PBR with this material has already been tested, improving productivity in microalgae (Sun et al., 2018). Temperature upsurge as a result of the absorbed wavelengths is still the biggest drawback, thus making it less adequate under conditions of intense illumination. Finally, thin film coatings destructively interfere with unwanted wavelengths instead of absorbing them, solving at the same time the overheating problems of glass coatings. These materials can easily be adapted for a PBR design, and they were recently tested on a cascade photobioreactor to cultivate high-density microalgal cultures for biodiesel production (Tan et al., 2020). They haven't been proved for PPB growth yet, but it has the potential to be a cost-efficient technology.

6.3.3. *Thermochromic materials*

One of the biggest problems in outdoor reactors is seasonal temperature changes. Specifically, in winter, low temperatures can cause a decrease in the metabolic efficiency and even freeze the reactor. Thermochromics materials have the capacity to change their transmittance and reflectance properties when subjected to temperature variations. They are usually employed as glazing materials in architecture, thus regulating the amount of light transmitted or reflected, depending on atmospheric temperature (Kamalisarvestani et al., 2013). They not only act as specific wavelength filters but also transmit more heat associated with IR and near-IR radiations at low temperatures (Granqvist & Niklasson, 2016). On the other hand, at high temperatures they reflect IR wavelengths, so they should be adapted into the reactor being easily removed during hot days. These properties can be perfectly adapted to the needs of PPB cultivation, through effective sunlight wavelength management and temperature regulation, but it needs an exhaustive experimental study to determine its suitability. No work has been published so far applying this technology.

6.4. *Bottlenecks on PPB retention systems for biopolymers production and potential measures to solve these bottlenecks*

PPB can be cultivated in reactors/tanks with varied configurations, where cells can freely circulate in the system or be retained through the presence of carriers, support materials or membranes (Chen et al., 2020). Biomass retention systems have been proposed as a means of reducing the system volume by concentrating cells, improving nutrient removal from the liquid stream and facilitating liquid separation from the biomass (Chen et al., 2020). Cells can be retained, for example, through the use of photo-anaerobic membrane bioreactors (PAnMBR) as proposed by Hülsen et al. (2016) for wastewater treatment with PPB. However, when PPB are operated for polymer production, the goal is quite the opposite from biomass retention. Instead, the aim is to stably remove as much biomass possible for polymer extraction. For up-scaled systems, it is even more important that a simple and direct access to the biomass is implemented with the lowest possible manual labour requirement. At the current technology development, suspended cells systems may be a better option, and while some settling issues may be pointed out, they may be minimized by the capacity of PPB to attach to particles present in the waste stream, forming granules. This can improve sludge settling and subsequent biomass recovery in PPB mixed culture systems.

6.4.1. Settling

One of the biggest operational costs in polymer extraction is the dewatering of biomass after its cultivation (Alloul et al., 2018). Settling is one of the most established methods for this purpose, but PPB collection is challenging due to their small size (0.2–4 μm), high electronegativity, and their stable suspension state in the culture medium (Chen et al., 2020). The easiest and cheapest would be gravitational settling but if not possible, membrane filtration can be performed followed by centrifugation (Alloul et al., 2018), although it will increase operating costs. Recently, in a pre-print, Cerruti et al. (2020) developed an enriched, concentrated and well-settling PPB mixed-culture, operated under SBR regime. This mixed-culture forms bio-aggregates with good settling properties. Settled biomass accounted for a 3-fold higher relative abundance of PNSB (80% as sum of *Rhodobacter*, *Rhodopseudomonas*, and *Blastochloris*) than the non-settled biomass (25%) with sedimentation G-flux of solids up to 4.7 kg h^{-1} m^{-2}. This should lead to an efficient solid to liquid separation, resulting in lowering costs for downstream processing by potentially reducing the need for ultrafiltration and centrifugation to concentrate the biomass.

6.4.2. Flocculation

Generally, flocculation has some advantages for industrial processes because of the simplicity of liquid/solid separation and the ease of cell mass retention in the reactor. However, flocculating photosynthetic bacteria have not yet been practically applied for industrial use due to their high cost associated. Some studies demonstrate how sodium, pH and light intensity affect flocculation for *Rhodobacter sphaeroides* (Lu et al., 2018). Watanabe et al. (1998) studied growth and flocculation of *Rhodovulum sp* and tried to improve it through addition of metal cations. The only study found about flocculation on PPB mixed cultures was published in 1983, and uses aluminum sulphate as a flocculant (Freedman et al., 1983). So, this is an open field of research in this process with PPB mixed cultures.

6.4.3. Photo-Granulation

From a bioprocess perspective, the high potential of granules to settle, facilitates the recovery and reuse of the active microbial biomass, and in the wastewater treatment context, the development of oxygenic photo-granules is a promising novelty (Abouhend et al., 2018).Also, granules are able to give way to IR light photons better than suspended biomass. Following the Beer-Lambert law, light absorbance will be enhanced with concentrated biomass, that make deeper bioreactors possible, improving volumetric productivity and reducing economic costs. Most studies have been performed on microalgae but the research on other type of microorganisms is attracting an increased interest and PPB ability to form granules has been already proven (Wilbanks et al., 2014). Controlling the formation of photo-granules is an open invitation to interdisciplinary collaborations between ecologists, bioprocess engineers and environmental scientists.

6.5. Homogeneity of the operation

Mixing is a crucial feature in the cultivation of any photosynthetic microorganism. Homogeneous mixing can reduce gradient of nutrients, pH, temperature and substrate in the reactor, while also preventing biomass settling, stagnant or dead zones and cell aggregation (Kumar et al., 2015). Besides, homogeneity ensures that all cells are equally exposed to the light and promotes mass transfer between phases. Mixing accounts for approximately 69% of the total utilities cost, in the range of 1.5–8.4 W m^{-3} (Martin et al., 2013). Therefore, unnecessarily mixing should be avoided, reducing the mixing velocity during night and even in winter season to minimize the operational cost. On a positive side, power consumption can be minimized under lower volume depths, which agrees well with PPB cultivation that deteriorates its growth over 20 cm of depth due to restricted illumination.

Several modifications in PBR have been proposed to tackle mixing costs, and demonstrated to enhance the biomass productivity, mixing efficiency and light penetration such as closed ponds, hybrid raceways, different flows through modular stack raceways or through unleveled baffled raceways (Kumar et al., 2015). Another important point to consider is inlets and outlet points on the reactor. A single inlet point can create deficient mixing and substrate gradient throughout the reactor (Enfors et al., 2001). Multiple inputs to the PBR should be considered, even more so in larger reactors.

6.6. Automatization of the system

Control systems play an important role in the development of bioreactors. In general, the main functions of a bioreactor control system include process control, monitoring, data gathering, and processing. The current trends on bioreactor control systems can be grouped into two aspects: some studies are focused on control strategies and algorithms adapted to bioreactor control systems (Steinwandter et al., 2019), while others mainly investigate the physical structure of the instrumental organization of bioreactor control systems (Wang et al., 2020).

PPB activity can be assessed by key performance indicators such as pH, absorbance, temperature and illumination, but also nutrient and substrate recovery rates and biomass productivity, for which suspended solids and nutrient concentrations must be measured. Although on-line sensors can monitor ammonium, nitrate, and suspended solids concentrations, they usually have higher capital and maintenance costs but are not always as reliable as expected. Therefore, they are often measured by time-consuming and expensive laboratory analyses (Foladori et al., 2018). The same happens with the production of biopolymers, since their intracellular concentration can only be measured in the laboratory, which amplifies the need for on-line monitoring strategies based on dynamic modeling dependent on indirect parameters such as pH, OD805/865 absorbances, illumination, temperature and the hydraulic retention time. But the complexity, inherent nonlinearity as well as high kinetic uncertainty, can make this approach very complicated. There are recent examples to control microalgae activity such as the works published by De-Luca et al. (2018) where a growth optimization based on uncertain weather forecasts is shown, or Robles et al. (2020) that demonstrated a community stabilization based on pH and dissolved oxygen. No work on control of PPB activity has been published yet. Control systems should be implemented as part of a SCADA (Supervisory, Control and Data Acquisition) program for supervision, data acquisition and equipment control. Reactor level control, feeding times, mixing, etc. can be output signals that can allow for better system optimization upon the dynamic model algorithm implementation.

7. Future directions on PHA production with PPB mixed cultures

On prospecting the transfer of the PHA producing technology with PPB mixed cultures to larger scale outdoor facilities it is important to mention that the two selection strategies currently applied to enrich the mixed culture in PHA producing PPB, feast and famine (FF) and permanent feast (PF), have very distinct modes of operation and requirements. Both strategies present strong points but also aspects that can be further improved. Table 3 compares, side by side, the main characteristics of the two strategies.

Looking to the characteristics of the FF strategy, the major factor that can hinder the technology transfer to large outdoor operation is the system dependence on microalgae to produce the oxygen that enables the PHA consumption during the famine phase. There is however margin to develop the FF technology by making the system independent of the microalgae presence. The microalgae elimination could further enrich the mixed culture in PPB while the advantageous features of the FF operation could be maintained. As previously mentioned, the system requires an electron acceptor during the famine phase, which can be provided through external aeration. Although it brings additional energy costs to the famine phase, the oxygen needs during the famine phase are substantially lower in comparison to the feast phase (2–5 times lower; (Third et al., 2003) and thus minimal aeration would be required. Therefore, future studies should evaluate if the costs incurred with aeration in the famine phase could be overcome by the following advantages: (i) the famine aerated phase could be schedule to the night time, and thus, the night would no longer be an idle period, but instead, could actively contribute to the FF process; (ii) with the famine phase occurring in the night, the feast phase could be extended up to the night time increasing the system PHA productivity and eventually eliminate the need for a secondary accumulator tank reactor (iii) aeration during the night famine phase allows the consumption of residual organic compounds that are not assimilated by PPB during the day anaerobic feast phase, permitting the discharge of a better quality effluent, (iv) aeration rates can be easily tuned to address the culture oxygen requirements at each night famine phase, maintaining the culture under very low oxygen concentrations (0.0–1.0 mg O_2/L) which decreases aeration energy costs and minimizes inhibition of PPB pigments expression, (v) the operational conditions lead to a microbial selection that favours the mixed culture enrichment in PPB that are more oxygen tolerant, which further overcomes eventual oxygen inhibitions due to the night aerated time. With these adaptations, the FF strategy would be the ideal choice for operation with feedstock that have highly reduced carbon compounds and high nutrients, since it does not rely in nutrient limitation for improved PHA accumulation. However, carbon dissipation will always occur in the famine phase during PHA consumption.

In relation to the PF strategy, its major challenge is related precisely to the feedstock composition. Since this strategy relies on the observance of anaerobic conditions, the presence of sugars and high sulphate concentrations in the feedstock can lead to the growth of a side-population composed of fermentative organisms or even of sulphate reducing bacteria (the latest supported by the highly reductive conditions promoted by the sugars). It is therefore important to control the sugar presence in the feedstock by implementing, for example, upstream fermentation processes. Furthermore, high nutrient concentrations in the feedstock can also challenge the production of high PHA content. In this situation, to favour the PHA storage over the growth, additional metabolic constraints may be needed in parallel to the selection under the permanent feast strategy. For example, adjustments of the food to microorganism ratio (F/M) may be a possible solution since F/M has been suggested to impact the dynamics of internal carbon flow during PHA storage in PPB systems (Fradinho et al., 2016). Also, the presence of eventual growth decouplers (aromatics, metals) in the feedstock may help limiting growth but not the PHA production activity (Puyol et al., 2020). For now, the PF selection strategy is suited for operation with feedstock that contain less reduced carbon sources (e.g. acetate, propionate) and low nutrient concentration (P, S) that allow the PHA storage over the growth. In comparison to the FF strategy, it has the advantage of high carbon recovery that approaches PF systems to CO_2 emission neutrality.

For the outdoor operation of PPB mixed cultures under the PF selection strategy, the current knowledge proposes the operation of two reactors/tanks: one for culture selection and growth with low light availability and a second for an external accumulation step under higher light availability. Decreasing implementation area by means of one single reactor operation would be a more economically attractive alternative. This raises the interesting, and not yet addressed question if simultaneous culture selection and high PHA production is possible. The culture would permanently present intrinsic high levels of PHA, but this would imply the system operation with higher light availability. A possible conflict with the culture selection could occur (as previously discussed) but also, a decrease of the culture overall photosynthetic efficiency could be observed (reduction of light harvesting complex levels due to acclimation to high light operation (Muzziotti et al., 2017). Further research can bring more insight to this challenging question.

Table 3
Characteristics and specific requirements of the feast and famine and the permanent feast strategies used for the selection of PHA producing PPB mixed cultures.

	Feast and Famine(PPB and microalgae)	Permanent Feast(PPB enriched culture)
Aeration	Aeration is eliminated since auto-oxygenation is provided by the microalgae; FF cycle must occur during the daytime.	Anaerobic operation, no aeration requirements.
Carbon recovery	High carbon recovery and conversion to PHA is achieved during the feast phase;PHA is partly dissipated as CO_2 during the famine phase.	Up to 100% carbon recovery (approaching the system to CO_2 neutrality) with high conversion to PHA.
Nutrient limitation	Carbon is the limiting nutrient that triggers PHA storage;No other nutrient limitation is required.	High PHA content is achieved under nutrient limitation, requiring feedstock with high carbon to nutrient ratios (e.g. high C:P or C:S ratios)
Electron balance	Oxygen availability during the famine phase reduces PPB internal redox stress allowing the uptake of more reduced substrates during the feast phase (e.g. butyric and valeric acids).	Anaerobic conditions induce consumption of less reduced substrates (e.g. acetate, propionate) to facilitate redox balance, leading to accumulation of less preferable compounds
OLR	Strict control in order to maintain a stable feast to famine ratio.	No famine phases; Organic carbon is continuously present simplifying system operation
Sugar presence in the feedstock	Oxygen presence inhibits fermentative metabolism.	Full fermentation of the feedstock must be assured to prevent fermentation processes that conflict with PHA production
Presence of Non-PHA producers	Although essential for oxygenation, microalgae decrease the overall PHA content in the biomass and may compete for carbon (e.g. acetate);	Microalgae are outcompeted; Fermenting organisms may grow if fermentable compounds are present in the feedstock (e.g. sugars, lactate)

8. Conclusions

The sustainable production of biopolymers from waste sources by mixed cultures of PPB is an emerging field that is facing several challenges. One key challenge is the integration of the complex metabolism of these organisms to allow control and optimization, which needs a dedicated modelling approach with novel components including energy, electrons and LHC. The use of current knowledge on mixed outdoor reactors based on PPB mixed cultures systems is crucial for achieving full-scale deployment of the technology. The need for advancing on upscaling challenges like improved sun illumination, biomass retention and collection, and feed control have promoted the development of specific projects focused on these challenges.

CRediT authorship contribution statement

J. Fradinho: Conceptualization, Methodology, Writing - original draft, Visualization, Supervision. **L.D. Allegue:** Writing - original draft, Visualization. **M. Ventura:** Writing - review & editing, Visualization. **J. A. Melero:** Writing - review & editing, Project administration, Funding acquisition. **M.A.M. Reis:** Writing - review & editing, Supervision, Project administration, Funding acquisition. **D. Puyol:** Conceptualization, Methodology, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Financial support from the Regional Government of Madrid through the project S2018/EMT-4344 BIOTRES-CM is gratefully acknowledged. D. Puyol wishes to thank the Spanish Ministry of Economy for the Ramon y Cajal grant. J. Fradinho and M. Reis acknowledge the support by the Applied Molecular Biosciences Unit - UCIBIO which is financed by national funds from FCT (UIDB/04378/2020). This work has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon2020 research and innovation program under grant agreement No.: 837998.

References:

Abouhend, A.S., McNair, A., Kuo-Dahab, W.C., Watt, C., Butler, C.S., Milferstedt, K., Hamelin, J., Seo, J., Gikonyo, G.J., El-Moselhy, K.M., Park, C., 2018. The Oxygenic Photogranule Process for Aeration-Free Wastewater Treatment. Environ. Sci. Technol. 52 (6), 3503–3511.

Albuquerque, M.G.E., Martino, V., Pollet, E., Avérous, L., Reis, M.A.M., 2011. Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: Effect of substrate composition and feeding regime on PHA productivity, composition and properties. J. Biotechnol. 151 (1), 66–76.

Allegue, L.D., Puyol, D., Melero, J.A., 2020. Novel approach for the treatment of the organic fraction of municipal solid waste: Coupling thermal hydrolysis with anaerobic digestion and photo-fermentation. Sci. Total Environ. 714, 136845.

Alloul, A., Ganigué, R., Spiller, M., Meerburg, F., Cagnetta, C., Rabaey, K., Vlaeminck, S. E., 2018. Capture–Ferment–Upgrade: A Three-Step Approach for the Valorization of Sewage Organics as Commodities. Environ. Sci. Technol. 52 (12), 6729–6742.

Alsiyabi, A., Immethun, C.M., Saha, R., 2019. Modeling the Interplay between Photosynthesis, CO 2 Fixation, and the Quinone Pool in a Purple Non-Sulfur Bacterium. Sci. Rep. 9 (1), 1–9.

Basak, N., Jana, A., Das, D., Saikia, D., 2014. Photofermentative molecular biohydrogen production by purple-non-sulfur (PNS) bacteria in various modes: The present progress and future perspective. Int. J. Hydrogen Energ. 39, 6853–6871.

Batstone, D.J., Hülsen, T., Oehmen, A., 2019. Metabolic modelling of mixed culture anaerobic microbial processes. Curr. Opin. Biotech. 57, 137–144.

Bayon-Vicente, G., Wattiez, R., Leroy, B., 2020. Global Proteomic Analysis Reveals High Light Intensity Adaptation Strategies and Polyhydroxyalkanoate Production in Rhodospirillum rubrum Cultivated With Acetate as Carbon Source. Front. Microbiol. 11.

Bioplastics, E., 2019. Bioplastics market data 2019. Global production capacities of bioplastics 2019-2024. European Bioplastics.

Blanken, W., Cuaresma, M., Wijffels, R.H., Janssen, M., 2013. Cultivation of microalgae on artificial light comes at a cost. Algal Res 2 (4), 333–340.

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

Carlozzi, P., Seggiani, M., Cinelli, P., Mallegni, N., Lazzeri, A.J.S., 2018. Photofermentative poly-3-Hydroxybutyrate production by rhodopseudomonas sp. S16-VOGS3 in a novel outdoor 70-L photobioreactor. Sustainability 10 (9), 3133.

Carvalho, V., Freitas, E., Fradinho, J., Reis, M., Oehmen, A., 2019. The effect of seed sludge on the selection of a photo-EBPR system. New Biotechnol. 49, 112–119.

Cerruti, M., Stevens, B., Ebrahimi, S., Alloul, A., Vlaeminck, S., Weissbrodt, D., 2020. Enriching and aggregating purple non-sulfur bacteria in an anaerobic sequencing-batch photobioreactor for nutrient capture from wastewater. bioRxiv 2020.01.08.899062.

Chen, C.-Y., Saratale, G., Lee, C.-M., Chen, P.-C., Chang, J.-S., 2008. Phototrophic hydrogen production in photobioreactors coupled with solar-energy-excited optical fibers. Int. J. Hydrogen Energ. 33, 6886–6895.

Chen, J., Wei, J., Ma, C., Yang, Z., Li, Z., Yang, X., Wang, M., Zhang, H., Hu, J., Zhang, C., 2020. Photosynthetic bacteria-based technology is a potential alternative to meet sustainable wastewater treatment requirement? Environ. Int. 137, 105417.

Comer, A.D., Abraham, J.P., Steiner, A.J., Korosh, T.C., Markley, A.L., Pfleger, B.F., 2020. Enhancing Photosynthetic Production of Glycogen-Rich Biomass for Use as a Fermentation Feedstock. Front. Energ Res. 8.

De-Luca, R., Trabuio, M., Barolo, M., Bezzo, F., 2018. Microalgae growth optimization in open ponds with uncertain weather data. Comput. Chem. Eng. 117, 410–419.

De Philippis, R., Ena, A., Guastiini, M., Sili, C., Vincenzini, M., 1992. Factors affecting poly-β-hydroxybutyrate accumulation in cyanobacteria and in purple non-sulfur bacteria. FEMS Microbiol. Lett. 103 (2), 187–194.

Desmidt, E., Ghyselbrecht, K., Zhang, Y., Pinoy, L., Van der Bruggen, B., Verstraete, W., Rabaey, K., Meesschaert, B., 2015. Global phosphorus scarcity and full-scale P-recovery techniques: a review. Crit. Rev. Environ. Sci. 45 (4), 336–384.

Dragone, G., Kerssemakers, A.A.J., Driessen, J.L.S.P., Yamakawa, C.K., Brumano, L.P., Mussatto, S.I., 2020. Innovation and strategic orientations for the development of advanced biorefineries. Bioresour. Technol. 302.

Egger, F., Hülsen, T., Tait, S., Batstone, D.J., 2020. Autotrophic sulfide removal by mixed culture purple phototrophic bacteria. Water Res. 182, 115896.

Enfors, S.O., Jahic, M., Rozkov, …, Manelius, Å., 2001. Physiological responses to mixing in large scale bioreactors. J. Biotechnol. 85(2), 175-185.

Foladori, P., Petrini, S., Andreottola, G., 2018. Evolution of real municipal wastewater treatment in photobioreactors and microalgae-bacteria consortia using real-time parameters. Chem. Eng. J. 345, 507–516.

Fradinho, J., Domingos, J., Carvalho, G., Oehmen, A., Reis, M., 2013a. Polyhydroxyalkanoates production by a mixed photosynthetic consortium of bacteria and algae. Bioresour. Technol. 132, 146–153.

Fradinho, J., Oehmen, A., Reis, M., 2019. Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating conditions. Int. J. Biol. Macromol. 126, 1085–1092.

Fradinho, J., Oehmen, A., Reis, M.A.M., 2013b. Effect of dark/light periods on the polyhydroxyalkanoate production of a photosynthetic mixed culture. Bioresour. Technol. 148C, 474–479.

Fradinho, J., Reis, M., Oehmen, A., 2016. Beyond feast and famine: Selecting a PHA accumulating photosynthetic mixed culture in a permanent feast regime. Water Res. 105, 421–428.

Fradinho, J.C., Oehmen, A., Reis, M.A., 2014. Photosynthetic mixed culture polyhydroxyalkanoate (PHA) production from individual and mixed volatile fatty acids (VFAs): substrate preferences and co-substrate uptake. J. Biotechnol. 185, 19–27.

Freedman, D., Koopman, B., Lincoln, E.P., 1983. Chemical and biological flocculation of purple sulphur bacteria in anaerobic lagoon effluent. J. Agri. Eng. Res. 28 (2), 115–125.

Granqvist, C., Niklasson, G., 2016. Thermochromic Oxide-Based Thin Films and Nanoparticle Composites for Energy-Efficient Glazings. Buildings 7 (4), 3.

Gupta, S., Pawar, S.B., Pandey, R., 2019. Current practices and challenges in using microalgae for treatment of nutrient rich wastewater from agro-based industries. Sci. Tot. Environ. 687, 1107–1126.

Hara, F., Akazawa, T., Kojima, K., 1973. Glycogen biosynthesis in Chromatium strain D: I. Characterization of glycogen1. Plant Cell Physiol. 14 (4), 737–745.

Hassan, M.A., Shirai, Y., Kusubayashi, N., Karim, M.I.A., Nakanishi, K., Hasimoto, K.J.J. o.F., Bioengineering, 1997. The production of polyhydroxyalkanoate from anaerobically treated palm oil mill effluent by Rhodobacter sphaeroides. J. Ferment. Bioeng. 83(5), 485-488.

Hill, S.K.E., Connell, R., Peterson, C., Hollinger, J., Hillmyer, M.A., Kortshagen, U., Ferry, V.E., 2019. Silicon Quantum Dot–Poly(methyl methacrylate) Nanocomposites with Reduced Light Scattering for Luminescent Solar Concentrators. ACS Photonics 6 (1), 170–180.

Hiraishi, A., Kitamura, H., 1985. Changes in the polyphosphate content of photosynthetically grown Rhodobacter sphaeroides due to nutrient limitation. J. Agr. Biol. Chem. 49 (11), 3343–3345.

Hiraishi, A., Yanase, A., Kitamura, H., 1991. Polyphosphate accumulation by Rhodobacter sphaeroides grown under different environmental conditions with special emphasis on the effect of external phosphate concentrations. Bull. Jap. Soc. Microb. Ecol. 6 (1), 25–32.

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

Hülsen, T., Batstone, D.J., Keller, J., 2014. Phototrophic bacteria for nutrient recovery from domestic wastewater. Water Res. 50, 18–26.

Hulsen, T., Lu, Y., Rodriguez, I., Segura, Y., Martinez, F., Puyol, D., Batstone, D.J., 2020. Anaerobic digestion of purple phototrophic bacteria - The release step of the partition-release-recover concept. Bioresour. Technol. 306, 123125.

Igarashi, R.Y., Meyer, C.R., 2000. Cloning and sequencing of glycogen metabolism genes from Rhodobacter sphaeroides 2.4.1. Expression and characterization of recombinant ADP-glucose pyrophosphorylase. Arch. Biochem. Biophys. 376 (1), 47–58.

Iliescu, S., Ilia, G., Plesu, N., Popa, A., Pascariu, A., 2006. Solvent and catalyst-free synthesis of polyphosphates. Green Chem. 8 (8), 727–730.

Jendrossek, D., 2009. Polyhydroxyalkanoate Granules Are Complex Subcellular Organelles (Carbonosomes). J. Bacteriol. 191 (10), 3195–3202.

Kamalisarvestani, M., Saidur, R., Mekhilef, S., Javadi, F.S., 2013. Performance, materials and coating technologies of thermochromic thin films on smart windows. Renew Sust. Energ. Rev. 26, 353–364.

Kemavongse, K., Prasertsan, P., Upaichit, A., Methacanon, P.J.W.J.o.M., Biotechnology, 2008. Poly-β-hydroxyalkanoate production by halotolerant Rhodobacter sphaeroides U7. World J Microbiol Biotechnol 24(10), 2073-2085.

Khatipov, E., Miyake, M., Miyake, J., Asada, Y.J.F.M.L., 1998. Accumulation of poly-β-hydroxybutyrate by Rhodobacter sphaeroides on various carbon and nitrogen substrates. FEMS Microbiol. Lett. 162 (1), 39–45.

Koku, H., Eroğlu, İ., Gündüz, U., Yücel, M., Türker, L., 2002. Aspects of the metabolism of hydrogen production by Rhodobacter sphaeroides. Int J Hydrogen Energ 27 (11–12), 1315–1329.

Koller, M., Marsalek, L., de Sousa Dias, M.M., Braunegg, G., 2017. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. N Biotechnol 37 (Pt A), 24–38.

Kumar, K., Mishra, S.K., Shrivastav, A., S. Park, M., Yanga, J.-W., 2015. Recent trends in the mass cultivation of algae in raceway ponds. Renew Sust Energ Rev 51, 875 - 885.

Lai, Y.C., Liang, C.M., Hsu, S.C., Hsieh, P.H., Hung, C.H., 2017. Polyphosphate metabolism by purple non-sulfur bacteria and its possible application on photo-microbial fuel cell. J. Biosci. Bioeng. 123 (6), 722–730.

Laurinavichene, T., Rákhely, G., Kovacs, K., Tsygankov, A., 2007. The effect of sulfur compounds on H2 evolution/consumption reactions, mediated by various hydrogenases, in the purple sulfur bacterium, Thiocapsa roseopersicina. Arch. Microbiol. 188, 403–410.

Laycock, B., Halley, P., Pratt, S., Werker, A., Lant, P., 2013. The chemomechanical properties of microbial polyhydroxyalkanoates. Prog. Polym. Sci. 38 (3–4), 536–583.

Liang, C.M., Hung, C.H., Hsu, S.C., Yeh, I.C., 2010. Purple nonsulfur bacteria diversity in activated sludge and its potential phosphorus-accumulating ability under different cultivation conditions. Appl. Microbiol. Biotechnol. 86 (2), 709–719.

Lu, H., Dong, S., Zhang, G., Han, T., Zhang, Y., Li, B., 2018. Enhancing the Auto-flocculation of Photosynthetic Bacteria to Realize Biomass Recovery in Brewery Wastewater Treatment. Environ. Technol. 40, 1–33.

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

Madigan, M.T., Gest, H., 1979. Growth of the photosynthetic bacterium Rhodopseudomonas capsulata chemoautotrophically in darkness with H2 as the energy source. J. Bacteriol. 137 (1), 524–530.

Marín, D., Posadas, E., García, D., Puyol, D., Lebrero, R., Muñoz, R., 2019. Assessing the potential of purple phototrophic bacteria for the simultaneous treatment of piggery wastewater and upgrading of biogas. Bioresour. Technol. 281, 10–17.

Martin, J.L., Granados, M., de Godos Crespo, I., Acien, G., Molina-Grima, E., Heaven, S., Banks, C., 2013. Oxygen transfer and evolution in microalgal culture in open raceways. Bioresour. Technol. 137C, 188–195.

McKinlay, J.B., Harwood, C.S., 2010. Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. Proc Nat Acad Sci 107 (26), 11669–11675.

Monroy, I., Buitron, G., 2020. Production of polyhydroxybutyrate by pure and mixed cultures of purple non-sulfur bacteria: A review. J. Biotechnol. 317, 39–47.

Muzziotti, D., Adessi, A., Faraloni, C., Torzillo, G., De Philippis, R., 2017. Acclimation strategy of Rhodopseudomonas palustris to high light irradiance. Microbiol. Res. 197, 49–55.

Nakajima, F., Kamiko, N., Yamamoto, K., 1997. Organic wastewater treatment without greenhouse gas emission by photosynthetic bacteria. Water Sci. Technol. 35 (8), 285–291.

Oliveira, C.S., Silva, C.E., Carvalho, G., Reis, M.A., 2017. Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: Feast and famine regime and uncoupled carbon and nitrogen availabilities. N Biotechnol 37, 69–79.

Pedrós-Alió, C., Mas, J., Guerrero, R., 1985. The influence of poly-β-hydoxybutyrate accumulation on cell volume and buoyant density in Alcaligenes eutrophus. Arch. Microbiol. 143, 178–184.

Pokorna, D., Zabranska, J., 2015. Sulfur-oxidizing bacteria in environmental technology. Biotechnol. Adv. 33 (6, Part 2), 1246–1259.

Puyol, D., Barry, E.M., Hülsen, T., Batstone, D.J., 2017. A mechanistic model for anaerobic phototrophs in domestic wastewater applications: Photo-anaerobic model (PAnM). Water Res. 116, 241–253.

Puyol, D., Hülsen, T., Padrino, B., Batstone, D., Martinez, F., Melero, J., 2020. Exploring the inhibition boundaries of mixed cultures of purple phototrophic bacteria for wastewater treatment in anaerobic conditions. Water Res. 183, 116057.

Puyol, D., Monsalvo, V., Marin, E., Rogalla, F., Melero, J., Martinez Castillejo, F., Hülsen, T., Batstone, D., 2019. Purple phototrophic bacteria as a platform to create the next generation of wastewater treatment plants: Energy and resource recovery. in: J.A. Olivares, D. Puyol, J.A. Melero, J. Dufour, (Eds.) Wastewater Treatment Residues as Resources for Biorefinery Products and Biofuels, Elsevier, pp. 255-280.

Raeisossadati, M., Moheimani, N.R., Parlevliet, D., 2019. Red and blue luminescent solar concentrators for increasing Arthrospira platensis biomass and phycocyanin productivity in outdoor raceway ponds. Bioresour. Technol. 291.

Rehm, B., 2010. Bacterial polymers: Biosynthesis, modifications and applications. Nat. Rev. Microbiol. 8, 578–592.

Reis, M., Albuquerque, M., Villano, M., Majone, M., 2011. Mixed culture processes for polyhydroxyalkanoate production from agro-industrial surplus/wastes as feedstocks. in: Compr Biotechnol, (Ed.) M. Moo-Young, Vol. 6, Academic Press, pp. 669-683.

Reusch, R.N., Sadoff, H.L., 1988. Putative structure and functions of a poly-beta-hydroxybutyrate/calcium polyphosphate channel in bacterial plasma membranes. Proc. Natl. Acad. Sci. 85 (12), 4176–4180.

Robles, Á., Capson-Tojo, G., Galès, A., Ruano, M.V., Sialve, B., Ferrer, J., Steyer, J.-P., 2020. Microalgae-bacteria consortia in high-rate ponds for treating urban wastewater: Elucidating the key state indicators under dynamic conditions. J. Environ. Manage. 261, 110244.

Sakarika, M., Spanoghe, J., Sui, Y., Wambacq, E., Grunert, O., Haesaert, G., Spiller, M., Vlaeminck, S.E., 2020. Purple non-sulphur bacteria and plant production: benefits for fertilization, stress resistance and the environment. Microb. Biotechnol. 13 (5), 1336–1365.

Scheer, H., 2006. An overview of chlorophylls and bacteriochlorophylls: biochemistry, biophysics, functions and applications. In: Chlorophylls and bacteriochlorophylls. Springer, pp. 1–26.

Sekar, K., Linker, S.M., Nguyen, J., Grunhagen, A., Stocker, R., Sauer, U., 2020. Bacterial Glycogen Provides Short-Term Benefits in Changing Environments. Appl. Environ. Microbiol. 86 (9).

Solovchenko, A., Verschoor, A.M., Jablonowski, N.D., Nedbal, L.J.B.a., 2016. Phosphorus from wastewater to crops: An alternative path involving microalgae. Biotechnol. Adv. 34(5), 550-564.

Steinwandter, V., Borchert, D., Herwig, C., 2019. Data science tools and applications on the way to Pharma 4.0. Drug Discov Today 24 (9), 1795–1805.

Sun, H., Zhao, W., Mao, X., Li, Y., Wu, T., Chen, F., 2018. High-value biomass from microalgae production platforms: strategies and progress based on carbon metabolism and energy conversion. Biotechnol. Biofuels 11 (1), 227.

Suzuki, I., 2001. Microbial leaching of metals from sulfide minerals. Biotechnol. Adv. 19 (2), 119–132.

Tan, C.H., Tan, X., Ho, S.-H., Lam, S.S., Show, P.L., Nguyen, T.H.P., 2020. Conceptual design of a hybrid thin layer cascade photobioreactor for microalgal biodiesel synthesis. Int. J. Energ. Res. 44 (12), 9757–9771.

Third, K.A., Newland, M., Cord-Ruwisch, R., 2003. The effect of dissolved oxygen on PHB accumulation in activated sludge cultures. Biotechnol. Bioeng. 82 (2), 238–250.

Timpmann, K., Chenchiliyan, M., Jalviste, E., Timney, J.A., Hunter, C.N., Freiberg, A., 2014. Efficiency of light harvesting in a photosynthetic bacterium adapted to different levels of light. BBA - Bioenergetics 1837 (10), 1835–1846.

Trüper, H.G., 1984. Phototrophic Bacteria and their Sulfur Metabolism. Stud. Inorg. Chem. 5, 367–382.

Tsuge, T., 2002. Metabolic improvements and use of inexpensive carbon sources in microbial production of polyhydroxyalkanoates. J. Biosci. Bioeng. 94 (6), 579–584.

Vainshtein, M.B., Gogotova, G.I., Heinritz, N.J., 1994. Removal of H2S by the purple sulphur bacterium Ectothiorhodospira shaposhnikovii. World J. Microbiol. Biotechnol. 10 (1), 110–111.

Wang, B., Wang, Z., Chen, T., Zhao, X., 2020. Development of Novel Bioreactor Control Systems Based on Smart Sensors and Actuators. Front. Bioeng. Biotechnol 8.

Watanabe, M., Shiba, H., Sasaki, K., Nakashimada, Y., Nishio, N., 1998. Promotion of growth and flocculation of a marine photosynthetic bacterium, Rhodovulum sp. by metal cations. Biotechnol. Lett. 20 (12), 1109–1112.

Wilbanks, E.G., Jaekel, U., Salman, V., Humphrey, P.T., Eisen, J.A., Facciotti, M.T., Buckley, D.H., Zinder, S.H., Druschel, G.K., Fike, D.A., Orphan, V.J., 2014. Microscale sulfur cycling in the phototrophic pink berry consortia of the S ippewissett S alt M arsh. Environ. Microbiol. 16 (11), 3398–3415.

Wondraczek, L., Gründler, A., Reupert, A., Wondraczek, K., Schmidt, M.A., Pohnert, G., Nolte, S., 2019. Biomimetic light dilution using side-emitting optical fiber for enhancing the productivity of microalgae reactors. Sci. Rep. 9 (1), 9600.

Zhang, J., Satti, A., Chen, X., Xiao, K., Sun, J., Yan, X., Liang, P., Zhang, X., Huang, X., 2015. Low-voltage electric field applied into MBR for fouling suppression: Performance and mechanisms. Chem. Eng. J. 273, 223–230.

Zhu, Y.S., Cook, D.N., Leach, F., Armstrong, G.A., Alberti, M., Hearst, J.E., 1986. Oxygen-regulated mRNAs for light-harvesting and reaction center complexes and for bacteriochlorophyll and carotenoid biosynthesis in Rhodobacter capsulatus during the shift from anaerobic to aerobic growth. J. Bacteriol. 168 (3), 1180–1188.