Creating value from purple phototrophic bacteria via single-cell protein production

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We hereby submit our manuscript entitled "Creating value from purple phototrophic bacteria via single-cell protein production " by Tim Hülsen, Andrew C. Barnes, Damien J. Batstone and Gabriel Capson-Tojo.

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Creating value from purple phototrophic bacteria via single-cell protein production

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Abstract

Purple phototrophic bacteria (PPB) technology for resource recovery is still in its infancy, but progress is occurring fast, and developments are accelerating. The generally higher photobioreactor costs have to be balanced with product revenue. The PPB biomass can be used as single cell protein where high protein contents can be complemented by value add components (*e.g.* pigments and ployhydroxyalkanoates), merging functionalities within a

single product. This has the potential to increase the product value and impact the economic feasibility, likely justifying higher capital costs for PPB photobioreactors for real life applications, with high future growth demand potential of the PPB product.

Highlights

- High capital costs need to be balanced by product value
- Feed value can be enhanced by pigments and biopolymers
- Microalgae experiences are not directly applicable to photo-bacteria
- Further scaled data needed from outdoor reactors

Graphical abstract

1 Introduction

Purple phototrophic bacteria (PPB) are a ubiquitous group of photosynthetic microbes with versatile metabolic capabilities. PPB perform anoxygenic photosynthesis, which does not produce oxygen because water does not serve as electron donor [1]. Instead, PPB use a diversity of organic donors, such as acetate and succinate, that serve as both carbon and electron source, and inorganic donors such as H_2, H_2S, or Fe^{2+}, for anaerobic photoheterotrophic and photoautotrophic growth respectively [2]. Phototrophic growth is driven by light harvesting (LH) complexes, (LHI and LHII) containing a range of carotenoids and bacteriochlorophylls (BChls), namely BChl a and/or BChl b. BChls absorb wavelengths in the near infra-red (NIR) spectrum, >850 and >1000 nm [3], while carotenoids have accessory light harvesting functions and also serve for photo-inhibition protection in the visible range (400-600 nm) [4]. The utilisation of light as energy source enables biomass yields close to unity on chemical oxygen demand (COD) basis during photoheterotrophic growth, and the exclusive capability of absorbing NIR wavelengths allows effective selection of PPB in non-sterile environments, such as wastewater [5].

The combination of effective NIR selection and enrichment with anaerobic photoheterotrophy and the consequent high biomass yields, have led to the re-emergence of PPB for environmental biotechnological applications, with a focus on resource recovery. Specifically, PPB have been applied for secondary/tertiary wastewater treatment, achieving the simultaneous removal of organics, nitrogen, and phosphorus from various wastewaters [2, 6]. Non-oxidative removal via biological assimilation enables the partitioning of soluble components (*e.g.* soluble COD, NH_4^+-N and PO_4^{3-}-P) into PPB biomass, a solid which can be recovered. This biomass is characterised by high crude protein (CP) contents at around 60% dry weight (DW), and varying amounts of carotenoids, BChls, vitamins and polyhydroxyalkanoates (PHAs) (Figure 1). Consequently, the generated biomass and its components are potential products, depending on quality which is mostly determined by the substrate used. Indeed, biomass has been tested as single cell protein (SCP) source, for example, as a feed additive and bulk ingredient in aquaculture [7, 8].

Photobioreactor (PBR) systems for biomass production and wastewater treatment have been researched extensively in the algae field [9]. It has been well established that the critical barriers are largely economic rather than technical. This is due to the increased capital and operational costs of PBRs compared to conventional treatment processes such as activated sludge systems [10, 11]. In fact, the monetisation of the biomass or related products is a prerequisite to balance the costs and to enable reasonable amortisation rates. Compared to microalgae, PPB products have received little attention and there are no commercial product. Nevertheless, if PPB are to be applied for wastewater treatment and resource recovery, PPB products have to generate revenue, necessitating the type of commercially focused research realised for microalgae products [12].

While it is an advantage for resource recovery to have biomass yields close to unity, it is a costly problem if this biomass cannot be valorised. The application as SCP has great potential due to the high protein content in PPB biomass, without requirement for extensive post-processing, as bulk biomass can be used directly. There is also potential value addition from the combination of PPB cell components. Other than proteins, PPB contain pigments (*i.e.* BChls and carotenoids) and PHA with potential additional functional benefits such as meat coloration and immuno-nutritional properties in, for example, aquaculture feeds [13, 14]. Whole cell PPB biomass with added functionalities will likely increase product value, improving the economic feasibility of the PPB technology.

Here, we summarise the most recent advances in the application of PPB biomass as SCP and its value-added potential due to the presence of other compounds, such as pigments or PHA. This review also addresses recent developments in cultivation of PPB in different configurations, as they are key to determine the production costs. Finally, recommendations for a successful implementation of PPB technology are given.

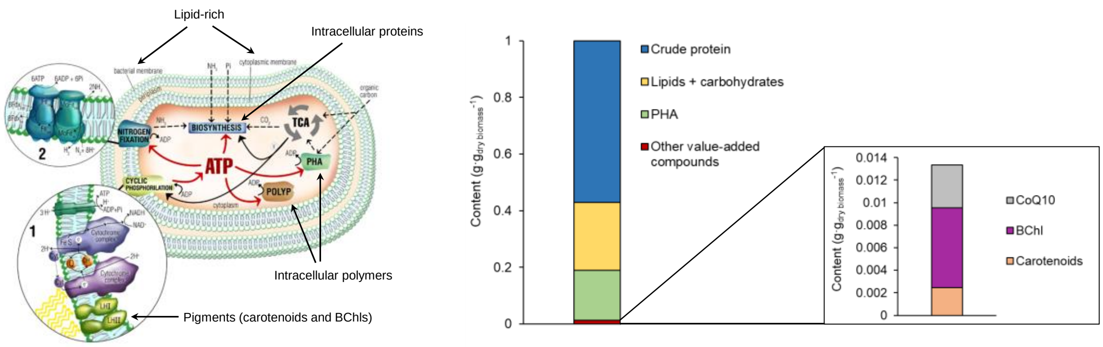

Figure 1: PPB cell diagram (left) and approximate product content (right; average values from the data presented in Capson-Tojo et al. (2020)).

2 PPB as single cell protein and added-value components for feeds

Production of fish and shellfish from aquaculture grew from around 10 million metric tonnes in 1987 to more than 80 million tonnes in 2017 [15]. Critically, the production of fed-fish, those fed on formulated compound diets, tripled between 2000 and 2017, while harvest of forage fish for fishmeal, the major protein source in aquaculture feeds, declined over the same period [15, 16]. This has resulted in a doubling of fishmeal price since 2020 [15] that, along with questions over sustainability, has driven demand for alternative protein ingredients for fish feeds from the manufacturers. Most plant or algae-based substitutes lack the correct balance of amino acids, contain anti-nutritional factors [17], and have insufficient protein content to formulate feeds for the most valuable farmed fish and shrimp that require >40% dietary protein . In contrast, PPB biomass is generally characterised by high crude protein contents (~60% DW), with a balanced amino acid profile, and including elevated levels of the essential amino acids cysteine and methionine [18], which are often added to aquaculture feed [19]. High protein contents and adequate amino acid profiles enable feed protein substitution for high value aquaculture species, such as salmonids carnivorous marine fish and shrimp that require up to 60% of protein in their diets [20]. In fact, PPB can substitute a major fraction of fishmeal in diets for Asian sea bass [7]. Fishmeal substitution with PPB was also tested in prawn feed trials, underlining the application of PPB biomass across diverse valuable taxa [8]. At lower inclusion rates, PPB inclusion in commercial fish (Nile tilapia, marble goby, barramundi and Tor tambroides [21, 22] and shrimp feed (Litopenaeus vannamei) increased survival, growth and resistance to challenges, compared with a control diet [23]. Theoretically, this makes PPB biomass as valuable as fishmeal (~1,500 USD·tonne^{-1}), although the critical price point to compete is expected to be lower than that of fishmeal. Here, immuno-nutritional components such as carotenoids, BChls, vitamins and PHA provide additional points-of-value (Figure 1). This is particularly the case for whole cell PPB products, which avoid costly substitution of extracted pure compounds to enhance vitamin, amino acid, and pigment levels. The value proposition for animals other than fish differs drastically, especially when low value protein sources such as soybean meal are substituted (470 USD·tonne^{-1}), which would hardly cover the production costs [24]. We therefore focus on PPB as source of SCP for aquaculture, with the substitution of fishmeal as primary target.

2.1 Secondary points-of-value

2.1.1 PPB pigments: carotenoids and bacteriochlorophylls

Carotenoids, especially xanthophylls, such as astaxanthin (ASX), are used as antioxidants, and as pigments for meat and skin coloration in commercial aqua feed. for shrimps and salmonids [25]. Incorporation of ASX in feed is recommended at levels of 50–100 mg·kg$_{feed}^{-1}$ in aquaculture, which represents between 10-18% of the total feed costs [26].

While PPB do not produce ASX, they do contain a range of other xanthophylls such as spirilloxanthin, lutein, okenone, spheroidene, rhodopin, *etc.*, besides a wide range of carotenes, including lycopene, neurosporene, *etc.* [27]. These might be relevant as meat colorants (more relevant for shrimps and to a lesser extent for fish) and/or as antioxidants and vitamin A precursors, which can improve the immune functions, liver structure and reproduction performance in fish and crustaceans [28]. Understanding the value of specific PPB pigments, other than lutein, is hampered by the fact that only a handful of analytical standards are available for chemical analysis. Consequently, development of methods for accurate carotenoid quantification, and evaluation of their effects as feed components is an essential prerequisite to extend the application spectrum and value of PPB biomass as a feed component. A similar problem applies to BChls in PPB, noting that chlorophyll a from cyanobacteria is a bioactive component, and part of the diet in herbivorous fish that comprise the majority of fish farmed for food [29]. Consequently, the potential value of pigments in PPB biomass has not yet been determined, a serious omission, as whole cell PPB products contain around 5-10 $mg \cdot g^{-1}$ total carotenoids and ~10-20 $mg \cdot g^{-1}$ BChls [2].

Intracellular carotenoids in whole cell products have substantial benefits over synthetic products, with the latter being more easily degraded by light, oxygen, acidity and temperature [26]. This stability may enable lower carotenoid inclusion rates in feed. Pigment content of PPB can be manipulated to a degree, for example via light intensity, but the natural selection of specific carotenoids in PPB biomass has not been reported [30]. Establishing how well the various PPB carotenoids are utilised by fish and shrimp, and potential impacts on animal growth performance are clear avenues for future research to enable full appraisal of benefits and potential revenues.

2.1.2 Polyhydroxyalkanoates (PHA)

Diseases remain a major constraint to growth and profitability in global aquaculture [15, 31]. While significant advances in disease control have been made, antibiotic use in some sectors of the industry remains high [32]. Set against a global "one health" initiative to reduce antimicrobial resistance in the environment and food supply to reduce the public health threat [31], alternative means of reducing or controlling diseases in aquaculture have high potential value. PHAs have been intensively studied for application as biodegradable polymers or bioplastics for packaging. In addition, PHA may have antimicrobial properties and immunomodulatory effects in fish and shrimps [13, 33]. In fact, PHA-rich additives in feed might increase resistance against common pathogens in aquaculture [34], a topic that has recently been reviewed [13]. Inclusion rates between 0.2-5%wt (weight) PHA generally increase the average weight gains, growth rates and survival rates of fish and shrimps in various growth stages, with species dependant effects [35]. These effects are at least partly caused by the microbial PHA degradation to short chain fatty acids (SCFA) in the gastrointestinal tract of the fish or shrimp. SCFA are commercially used as antimicrobial bio-agents in commercial animal feed [36].

Mixed and pure PPB cultures readily produce PHA [37] and can accumulate up to 82%wt under nutrient limiting conditions in dedicated processes [38]. For feed applications, high PHA contents are not required to reach the desired PHA inclusion rates for feed applications (usually around 0.2-5%wt). Non-PHA directed PPB growth resulted in around 2.5%wt of PHA [7], which may be sufficient to add value to the PPB biomass. With limited extra effort, small amounts of PHA can be generated in a PPB process. Intracellular PHA is naturally bio-encapsulated, which enables effective delivery to the gastrointestinal tract of the target species, improving feed palatability (no PHA odour), while minimising losses through leaching during feeding. The amorphous character of intracellular PHA also improves the biotic and abiotic degradability compared to crystalline PHA [39]. This might be a more realistic application of PPB-generated PHA, compared to pure PHA production from PPB, which incurs substantial extraction and purification costs and has production rates that are orders of magnitude lower (~0.5 g $PHA \cdot L^{-1} \cdot d^{-1}$) [2] compared to aerobic platforms (>10 g $PHA \cdot L^{-1} \cdot d^{-1}$) [40]. Instead, PHA within the PPB biomass, together with the protein and pigment contents, can substantially increase the biomass values, thanks to the potential benefits of combined functionalities within a single product. There is currently no research about the combined effects on performance of aquatic species fed intracellular PHA from PPB.

3 PPB cultivation systems

For microalgae cultivation, the most commonly applied systems are high rate algae ponds (HRAPs), with some niche applications of closed PBRs for high value products from sterile substrates [41]. The costs and life cycle analyses for microalgae systems have been detailed in several reviews, *e.g.* [42].

Economic analyses for PPB mediated systems are basic, and usually rely on artificially illuminated, small-scale lab research, which hardly captures the true picture of a scaled, outdoors PPB wastewater treatment plant [24, 43]. The main problem at the moment is that scaled capital and operational costs have not been detailed, as studies in outdoors, scalable systems are absent from the literature. Therefore, accurate datasets evaluating annual performances, including areal productivities, removal efficiencies and rates, as well as the quality and consistency of the produced biomass and its applicability as product, are missing.

PPB reactor technology is still in its infancy. Recent progress is limited to medium-scale laboratory and field reactors (<100 L) employing artificial illumination in controlled environments. There is no consensus on the reactor design, and several configurations have been proposed. These include closed PBRs (flat plate, tubular, cylindrical, sequencing batch reactors, gas-lift, *etc.*) and reconfigured HRAP [2].

One might think that 60 years of algal research would have paved the way for PPB, but results from algal research cannot directly be translated to PPB systems. This is a consequence of the profound differences among these two microbial groups and include; (i) NIR *vs.* visible (VIS) light uptake (ii) anaerobic *vs.* aerobic phototrophic growth (iii) photoheterotrophic *vs.* photoautotrophic growth which necessitate organic carbon *vs.* CO_2 supply. Due to the COD requirements, PPB are fundamentally more suited to secondary treatment, whereas algae systems are well suited to tertiary applications. In addition, higher biomass concentrations can be achieved in PPB reactors, and PPB grow faster than algae [2]. These aspects affect the process fundamentally, modifying the light distribution through the reactor, potential microbial synergies and competitions, the growth kinetics, reactor design and mixing, biomass contents, and various design parameters, such as hydraulic retention time (HRT), sludge retention time (SRT) and organic loading rate (OLR). Open ponds have lower volumetric cost per unit than closed PBRs, but the combination of poor NIR penetration, high PPB biomass concentrations, and the requirement to limit oxygen input (see below) complicate their use. Recent studies confirm the issue of light limitation in ponds, with light-limited processes at light paths (pond depth) of 10, 15 and 20 cm (surface to volume ratio (S/V) of 5-10 $m^2 \cdot m^{-3}$) [44, 45]. Sepúlveda-Muñoz, Ángeles [45] found an improved performance with a light path of 7.5 cm when combined with a light intensity increase from 100–200 $W \cdot m^{-2}$, confirming that light was limiting. A 15 cm deep horizontal PBR (simulating a pond) required HRTs of 4-11 d, which are much higher than those commonly applied (PPB have phototrophic growth rates of 2-4 d^{-1} [46-48]). This further confirm light limitation at these conditions. Such short light transfer distances imply large footprints (land areas), which increases cost and oxygen transfer into the liquid. Excessive oxygen transfer (i) inhibits PPB pigment synthesis [49] and allows the growth of aerobic heterotrophs. This has the potential to result in complete out-competition of PPB [50]. This will most certainly result in mixed cultures, inconsistent product contents, and reduced value and may be referred to as PPBALBAZOD (Purple Phototrophic Bacteria, Algae, Bacteria, Zooplankton and Detritus).

Flat plate PBRs might be an elegant alternative to ponds, reducing the required land area and the oxygen transfer, while effectively doubling the light path (*i.e.* at the same pond depth and PBR thickness, the light path is double in the PBR due to illumination from both sides) and increasing the illuminated surface to volume ratio, albeit at higher capital cost (Figure 2). Costs can be further decreased by low energy mixing options, no need for CO_2 supply or O_2 stripping, and no cooling requirements. PPB tolerate up to 55°C and daily temperature fluctuations between 20-30°C [51]. Any viable PBR must be run outdoors unless energy for artificial illumination is free (or exceptionally cheap) [2]. Mixing, harvesting, up-concentration, drying and sterilisation costs have to be added. To date, there is no realistic value assessing these costs as long term outdoor data are missing, and economic studies and life cycle analysis are based on lab results [24, 43, 46]. The maximum allowable production costs cannot exceed the sum of wastewater treatment (*i.e.* discharge savings) and the product revenue to enable a reasonable payback time for the PPB technology. Mixing is an issue still to be addressed. Inert gas mixing and return liquid mixing is energetically expensive, and paddle wheels increase oxygen transfer [52]. PPB can be harvested as biofilms from illuminated submerged surfaces at >10% dry solids [5, 7]. Formation of flocculant or granular biomass also has the potential to reduce harvesting costs [53, 54], and might improve the product consistency over time.

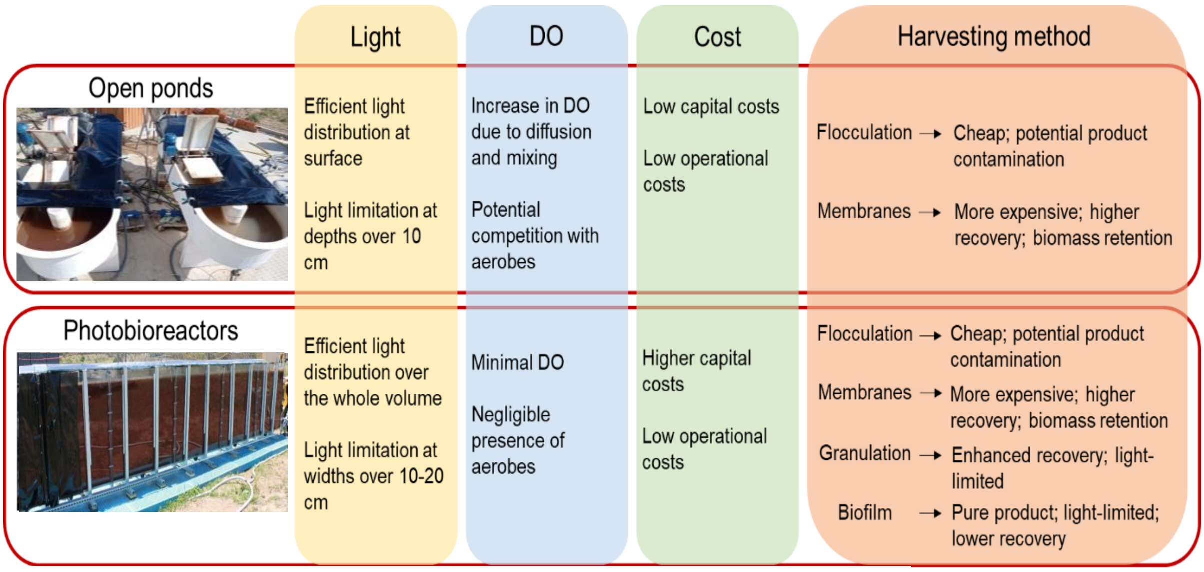

Figure 2: Qualitative comparison of an open pond and a flat plate PBR for PPB cultivation. Pictures from (top) phototrophic purple bacteria ponds of the Universidad Rey Juan Carlos and Aqualia and (bottom) flat plate PBR from the University of Queensland.

4 Further research

In order to advance and finally implement the PPB technology in the real world, research needs to move to relevant-scale outdoor units. These units will enable the determination of the impact that biotic and abiotic factors have on the wastewater treatment performance, as well as on the PPB cultivation and the product quality on an annual basis. This will enable an economic evaluation, while producing large quantities of PPB biomass that can be used for feed trials. Further fundamental research is needed on the manipulation of pigment and PHA contents, including validating their effect via dedicated feed trials. This should include feed manufacturing as well as nutritional aspects, and requires a multidisciplinary team of researchers. High value fish and shrimp is a clear initial target to maximise the value of the product. However, use as feed is overall the most economically viable use of PPB biomass, and has already been validated in the more expensive algae area .

5 Conclusion

PPB technology is still in its infancy, but progress is occurring fast, and developments are accelerating. The economics of different PPB cultivation systems have to be evaluated and the value of PPB biomass as product will play a major role in the overall feasibility and subsequent realisation of a PPB platform. The potential value add of the product will impact the overall feasibility, likely justifying higher capital costs for closed photo bioreactors for real life applications, with high future growth demand potential of the PPB product.

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*Declaration of Interest Statement

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