



HAL
open science

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

Tim Hülsen, Andrew C Barnes, Damien J Batstone, Gabriel Capson-Tojo

► **To cite this version:**

Tim Hülsen, Andrew C Barnes, Damien J Batstone, Gabriel Capson-Tojo. Creating value from purple phototrophic bacteria via single-cell protein production. *Current Opinion in Biotechnology*, 2022, 76, 10.1016/j.copbio.2022.102726 . hal-03777889

HAL Id: hal-03777889

<https://hal.inrae.fr/hal-03777889>

Submitted on 13 Aug 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Current Opinion in Biotechnology

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

--Manuscript Draft--

Short Title:	Phototrophic bacteria as single cell protein
Keywords:	Purple phototrophic bacteria; single cell protein; aquafeed; protein; polyhydroxyalkanoates; carotenoids
Corresponding Author:	Tim Hülsen UQ AWMC: The University of Queensland Advanced Water Management Centre AUSTRALIA
Corresponding Author's Institution:	UQ AWMC: The University of Queensland Advanced Water Management Centre
Corresponding Author E-Mail:	t.huelsen@awmc.uq.edu.au
First Author:	Tim Hülsen
Order of Authors:	Tim Hülsen Andrew C. Barnes Damien J. Batstone Gabriel Capson-Tojo
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 polyhydroxyalkanoates), 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.
Author Comments:	



Editor, Current opinion in Biotechnology

Brisbane, 01 November 2021

Advanced Water Management Centre
The University of Queensland
Level 4, Gehrmann Bldg (60)
Brisbane, QLD, 4072, Australia
Telephone +61 7 3365 4730
Facsimile +61 7 3365 4726
Internet www.awmc.uq.edu.au

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.

Kind regards,

Dr. Tim Hülsen

Senior Research Fellow

Australian Centre for Water and Environmental Biotechnology (formerly AWMC)The University
of Queensland

Level 4, Gehrmann Building (60)

Brisbane QLD 4072 Australia

Phone: +61 (0)450760068

t.huelsen@awmc.uq.edu.au

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

2 Tim Hülsen ^{a,*}, Andrew C. Barnes ^b, Damien J. Batstone ^a, Gabriel Capson-Tojo ^{a,c}

3

4 ^a Advanced Water Management Centre, Gehrmann Building, The University of Queensland,
5 Brisbane, Queensland 4072, Australia

6 ^b School of Biological Sciences and Centre for Marine Science, The University of Queensland,
7 Brisbane, Queensland, 4072, Australia

8 ^c CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela,
9 15782 Santiago de Compostela, Galicia, Spain

10

11 *Corresponding Author:

12 Tim Hülsen

13 Advanced Water Management Centre

14 The University of Queensland

15 St Lucia, Brisbane, 4072, Australia

16 Phone: +61 (0)7 33467209

17 Fax: +61(0)733654726

18 E-mail: t.huelsen@awmc.uq.edu.au

19

20 **Abstract**

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

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

29

30 **Highlights**

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

35

36 **Graphical abstract**



38 **1 Introduction**

39 Purple phototrophic bacteria (PPB) are a ubiquitous group of photosynthetic microbes with
40 versatile metabolic capabilities. PPB perform anoxygenic photosynthesis, which does not
41 produce oxygen because water does not serve as electron donor [1]. Instead, PPB use a diversity
42 of organic donors, such as acetate and succinate, that serve as both carbon and electron source,
43 and inorganic donors such as H_2 , H_2S , or Fe^{2+} , for anaerobic photoheterotrophic and

44 photoautotrophic growth respectively [2]. Phototrophic growth is driven by light harvesting
45 (LH) complexes, (LHI and LHII) containing a range of carotenoids and bacteriochlorophylls
46 (BChls), namely BChl a and/or BChl b. BChls absorb wavelengths in the near infra-red (NIR)
47 spectrum, >850 and >1000 nm [3], while carotenoids have accessory light harvesting functions
48 and also serve for photo-inhibition protection in the visible range (400-600 nm) [4]. The
49 utilisation of light as energy source enables biomass yields close to unity on chemical oxygen
50 demand (COD) basis during photoheterotrophic growth, and the exclusive capability of
51 absorbing NIR wavelengths allows effective selection of PPB in non-sterile environments, such
52 as wastewater [5].

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

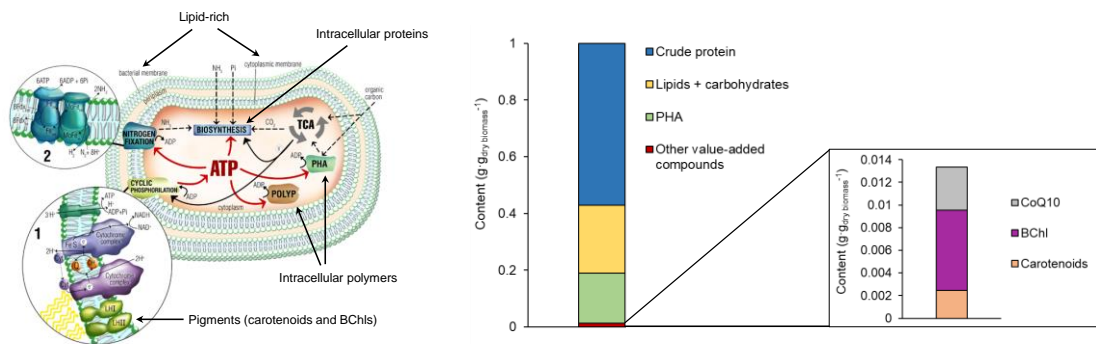
66 Photobioreactor (PBR) systems for biomass production and wastewater treatment have been
67 researched extensively in the algae field [9]. It has been well established that the critical barriers
68 are largely economic rather than technical. This is due to the increased capital and operational

69 costs of PBRs compared to conventional treatment processes such as activated sludge systems
70 [10, 11]. In fact, the monetisation of the biomass or related products is a prerequisite to balance
71 the costs and to enable reasonable amortisation rates. Compared to microalgae, PPB products
72 have received little attention and there are no commercial product. Nevertheless, if PPB are to
73 be applied for wastewater treatment and resource recovery, PPB products have to generate
74 revenue, necessitating the type of commercially focused research realised for microalgae
75 products [12].

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

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

90



91

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

94

95 2 PPB as single cell protein and added-value components for feeds

96 Production of fish and shellfish from aquaculture grew from around 10 million metric tonnes
97 in 1987 to more than 80 million tonnes in 2017 [15]. Critically, the production of fed-fish, those
98 fed on formulated compound diets, tripled between 2000 and 2017, while harvest of forage fish
99 for fishmeal, the major protein source in aquaculture feeds, declined over the same period [15,
100 16]. This has resulted in a doubling of fishmeal price since 2020 [15] that, along with questions
101 over sustainability, has driven demand for alternative protein ingredients for fish feeds from
102 the manufacturers. Most plant or algae-based substitutes lack the correct balance of amino
103 acids, contain anti-nutritional factors [17], and have insufficient protein content to formulate
104 feeds for the most valuable farmed fish and shrimp that require >40% dietary protein . In
105 contrast, PPB biomass is generally characterised by high crude protein contents (~60% DW),
106 with a balanced amino acid profile, and including elevated levels of the essential amino acids
107 cysteine and methionine [18], which are often added to aquaculture feed [19]. High protein
108 contents and adequate amino acid profiles enable feed protein substitution for high value
109 aquaculture species, such as salmonids carnivorous marine fish and shrimp that require up to
110 60% of protein in their diets [20]. In fact, PPB can substitute a major fraction of fishmeal in
111 diets for Asian sea bass [7]. Fishmeal substitution with PPB was also tested in prawn feed trials,

112 underlining the application of PPB biomass across diverse valuable taxa [8]. At lower inclusion
113 rates, PPB inclusion in commercial fish (Nile tilapia, marble goby, barramundi and Tor
114 tambroides [21, 22] and shrimp feed (*Litopenaeus vannamei*) increased survival, growth and
115 resistance to challenges, compared with a control diet [23]. Theoretically, this makes PPB
116 biomass as valuable as fishmeal ($\sim 1,500 \text{ USD}\cdot\text{tonne}^{-1}$), although the critical price point to
117 compete is expected to be lower than that of fishmeal. Here, immuno-nutritional components
118 such as carotenoids, BChls, vitamins and PHA provide additional points-of-value (Figure 1).
119 This is particularly the case for whole cell PPB products, which avoid costly substitution of
120 extracted pure compounds to enhance vitamin, amino acid, and pigment levels. The value
121 proposition for animals other than fish differs drastically, especially when low value protein
122 sources such as soybean meal are substituted ($470 \text{ USD}\cdot\text{tonne}^{-1}$), which would hardly cover
123 the production costs [24]. We therefore focus on PPB as source of SCP for aquaculture, with
124 the substitution of fishmeal as primary target.

125 **2.1 Secondary points-of-value**

126 **2.1.1 PPB pigments: carotenoids and bacteriochlorophylls**

127 Carotenoids, especially xanthophylls, such as astaxanthin (ASX), are used as antioxidants, and
128 as pigments for meat and skin coloration in commercial aqua feed. for shrimps and salmonids
129 [25]. Incorporation of ASX in feed is recommended at levels of $50\text{--}100 \text{ mg}\cdot\text{kg}_{\text{feed}}^{-1}$ in
130 aquaculture, which represents between 10-18% of the total feed costs [26].

131 While PPB do not produce ASX, they do contain a range of other xanthophylls such as
132 spirilloxanthin, lutein, okenone, spheroidene, rhodopin, *etc.*, besides a wide range of carotenes,
133 including lycopene, neurosporene, *etc.* [27]. These might be relevant as meat colorants (more
134 relevant for shrimps and to a lesser extent for fish) and/or as antioxidants and vitamin A
135 precursors, which can improve the immune functions, liver structure and reproduction
136 performance in fish and crustaceans [28]. Understanding the value of specific PPB pigments,

137 other than lutein, is hampered by the fact that only a handful of analytical standards are
138 available for chemical analysis. Consequently, development of methods for accurate carotenoid
139 quantification, and evaluation of their effects as feed components is an essential prerequisite to
140 extend the application spectrum and value of PPB biomass as a feed component. A similar
141 problem applies to BChls in PPB, noting that chlorophyll a from cyanobacteria is a bioactive
142 component, and part of the diet in herbivorous fish that comprise the majority of fish farmed
143 for food [29]. Consequently, the potential value of pigments in PPB biomass has not yet been
144 determined, a serious omission, as whole cell PPB products contain around 5-10 mg·g⁻¹ total
145 carotenoids and ~10-20 mg·g⁻¹ BChls [2].

146 Intracellular carotenoids in whole cell products have substantial benefits over synthetic
147 products, with the latter being more easily degraded by light, oxygen, acidity and temperature
148 [26]. This stability may enable lower carotenoid inclusion rates in feed. Pigment content of PPB can be manipulated
149 to a degree, for example via light intensity, but the natural selection of specific carotenoids in
150 PPB biomass has not been reported [30]. Establishing how well the various PPB carotenoids
151 are utilised by fish and shrimp, and potential impacts on animal growth performance are clear
152 avenues for future research to enable full appraisal of benefits and potential revenues.

153 **2.1.2 Polyhydroxyalkanoates (PHA)**

154 Diseases remain a major constraint to growth and profitability in global aquaculture [15, 31].
155 While significant advances in disease control have been made, antibiotic use in some sectors
156 of the industry remains high [32]. Set against a global "one health" initiative to reduce
157 antimicrobial resistance in the environment and food supply to reduce the public health threat
158 [31], alternative means of reducing or controlling diseases in aquaculture have high potential
159 value. PHAs have been intensively studied for application as biodegradable polymers or
160 bioplastics for packaging. In addition, PHA may have antimicrobial properties and
161 immunomodulatory effects in fish and shrimps [13, 33]. In fact, PHA-rich additives in feed

162 might increase resistance against common pathogens in aquaculture [34], a topic that has
163 recently been reviewed [13]. Inclusion rates between 0.2-5%wt (weight) PHA generally
164 increase the average weight gains, growth rates and survival rates of fish and shrimps in various
165 growth stages, with species dependant effects [35]. These effects are at least partly caused by
166 the microbial PHA degradation to short chain fatty acids (SCFA) in the gastrointestinal tract
167 of the fish or shrimp. SCFA are commercially used as antimicrobial bio-agents in commercial
168 animal feed [36].

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

186 **3 PPB cultivation systems**

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



191 Economic analyses for PPB mediated systems are basic, and usually rely on artificially
192 illuminated, small-scale lab research, which hardly captures the true picture of a scaled,
193 outdoors PPB wastewater treatment plant [24, 43]. The main problem at the moment is that
194 scaled capital and operational costs have not been detailed, as studies in outdoors, scalable
195 systems are absent from the literature. Therefore, accurate datasets evaluating annual
196 performances, including areal productivities, removal efficiencies and rates, as well as the
197 quality and consistency of the produced biomass and its applicability as product, are missing.
198 PPB reactor technology is still in its infancy. Recent progress is limited to medium-scale
199 laboratory and field reactors (<100 L) employing artificial illumination in controlled
200 environments. There is no consensus on the reactor design, and several configurations have
201 been proposed. These include closed PBRs (flat plate, tubular, cylindrical, sequencing batch
202 reactors, gas-lift, *etc.*) and reconfigured HRAP [2].

203 One might think that 60 years of algal research would have paved the way for PPB, but results
204 from algal research cannot directly be translated to PPB systems. This is a consequence of the
205 profound differences among these two microbial groups and include; (i) NIR *vs.* visible (VIS)
206 light uptake (ii) anaerobic *vs.* aerobic phototrophic growth (iii) photoheterotrophic *vs.*
207 photoautotrophic growth which necessitate organic carbon *vs.* CO₂ supply. Due to the COD
208 requirements, PPB are fundamentally more suited to secondary treatment, whereas algae
209 systems are well suited to tertiary applications. In addition, higher biomass concentrations can
210 be achieved in PPB reactors, and PPB grow faster than algae [2]. These aspects affect the

211 process fundamentally, modifying the light distribution through the reactor, potential microbial
212 synergies and competitions, the growth kinetics, reactor design and mixing, biomass contents,
213 and various design parameters, such as hydraulic retention time (HRT), sludge retention time
214 (SRT) and organic loading rate (OLR). Open ponds have lower volumetric cost per unit than
215 closed PBRs, but the combination of poor NIR penetration, high PPB biomass concentrations,
216 and the requirement to limit oxygen input (see below) complicate their use. Recent studies
217 confirm the issue of light limitation in ponds, with light-limited processes at light paths (pond
218 depth) of 10, 15 and 20 cm (surface to volume ratio (S/V) of 5-10 m²·m⁻³) [44, 45]. Sepúlveda-
219 Muñoz, Ángeles [45] found an improved performance with a light path of 7.5 cm when
220 combined with a light intensity increase from 100–200 W·m⁻², confirming that light was
221 limiting. A 15 cm deep horizontal PBR (simulating a pond) required HRTs of 4-11 d, which
222 are much higher than those commonly applied (PPB have phototrophic growth rates of 2-4 d⁻¹
223 [46-48]). This further confirm light limitation at these conditions. Such short light transfer
224 distances imply large footprints (land areas), which increases cost and oxygen transfer into the
225 liquid. Excessive oxygen transfer (i) inhibits PPB pigment synthesis [49] and allows the growth
226 of aerobic heterotrophs. This has the potential to result in complete out-competition of PPB
227 [50]. This will most certainly result in mixed cultures, inconsistent product contents, and
228 reduced value and may be referred to as PPBALBAZOD (Purple Phototrophic Bacteria, Algae,
229 Bacteria, Zooplankton and Detritus).

230 Flat plate PBRs might be an elegant alternative to ponds, reducing the required land area and
231 the oxygen transfer, while effectively doubling the light path (*i.e.* at the same pond depth and
232 PBR thickness, the light path is double in the PBR due to illumination from both sides) and
233 increasing the illuminated surface to volume ratio, albeit at higher capital cost (Figure 2). Costs
234 can be further decreased by low energy mixing options, no need for CO₂ supply or O₂ stripping,
235 and no cooling requirements. PPB tolerate up to 55°C and daily temperature fluctuations

236 between 20-30°C [51]. Any viable PBR must be run outdoors unless energy for artificial
 237 illumination is free (or exceptionally cheap) [2]. Mixing, harvesting, up-concentration, drying
 238 and sterilisation costs have to be added. To date, there is no realistic value assessing these costs
 239 as long term outdoor data are missing, and economic studies and life cycle analysis are based
 240 on lab results [24, 43, 46]. The maximum allowable production costs cannot exceed the sum
 241 of wastewater treatment (*i.e.* discharge savings) and the product revenue to enable a reasonable
 242 payback time for the PPB technology. Mixing is an issue still to be addressed. Inert gas mixing
 243 and return liquid mixing is energetically expensive, and paddle wheels increase oxygen transfer
 244 [52]. PPB can be harvested as biofilms from illuminated submerged surfaces at >10% dry solids
 245 [5, 7]. Formation of flocculant or granular biomass also has the potential to reduce harvesting
 246 costs [53, 54], and might improve the product consistency over time.

	Light	DO	Cost	Harvesting method
Open ponds 	Efficient light distribution at surface Light limitation at depths over 10 cm	Increase in DO due to diffusion and mixing Potential competition with aerobes	Low capital costs Low operational costs	Flocculation → Cheap; potential product contamination Membranes → More expensive; higher recovery; biomass retention
Photobioreactors 	Efficient light distribution over the whole volume Light limitation at widths over 10-20 cm	Minimal DO Negligible presence of aerobes	Higher capital costs Low operational costs	Flocculation → Cheap; potential product contamination Membranes → More expensive; higher recovery; biomass retention Granulation → Enhanced recovery; light-limited Biofilm → Pure product; light-limited; lower recovery

247
 248 Figure 2: Qualitative comparison of an open pond and a flat plate PBR for PPB cultivation.
 249 Pictures from (top) phototrophic purple bacteria ponds of the Universidad Rey Juan Carlos and
 250 Aqualia and (bottom) flat plate PBR from the University of Queensland.

251 4 Further research

252 In order to advance and finally implement the PPB technology in the real world, research needs

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

263

264 **5 Conclusion**

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

271

272 **Acknowledgment**

273 Tim Hülsen is grateful to The Queensland Government, GHD, Ridley, Aquatec Maxcon and
274 Ingham for their financial support as part of an Advanced Queensland Industry Research
275 Fellowship. Gabriel Capson-Tojo wants to acknowledge the Xunta de Galicia for his
276 postdoctoral fellowship (ED481B-2018/017).

277

278 **6 References**

279 1. Ozaki K, Thompson KJ, Simister RL, Crowe SA, Reinhard CT. Anoxygenic
280 photosynthesis and the delayed oxygenation of Earth's atmosphere. *Nature*
281 *Communications*. 2019 10:3026.

282 ●●2. Capson-Tojo G, Batstone DJ, Grassino M, Vlaeminck SE, Puyol D, Verstraete W,
283 Kleerebezem R, Oehmen A, Ghimire A, Pikaar I, Lema J, Hülsen T. Purple phototrophic
284 bacteria for resource recovery: Challenges and opportunities. *Biotechnology Advances*.
285 2020 43:107567. *Critical review about the PPB reactor technology to set the*
286 *requirements for future research in phototrophic bacteria research, application and*
287 *scale-up. It further reviews PPB biomass components, compositions and contents and*
288 *give a value range for each.*

289 3. Nagashima KVP, Nagashima S, Kitashima M, Inoue K, Madigan MT, Kimura Y, et al.
290 Photosynthetic Growth and Energy Conversion in an Engineered Phototroph Containing
291 Thermochromatium tepidum Light-Harvesting Complex 1 and the Rhodobacter
292 sphaeroides Reaction Center Complex. *Biochemistry*. 2021 60:2685-90.

293 4. Seto R, Takaichi S, Kurihara T, Kishi R, Honda M, Takenaka S, et al. Lycopene-Family
294 Carotenoids Confer Thermostability on Photocomplexes from a New Thermophilic
295 Purple Bacterium. *Biochemistry*. 2020 59:2351-8.

296 5. Hülsen T, Sander EM, Jensen PD, Batstone DJ. Application of purple phototrophic
297 bacteria in a biofilm photobioreactor for single cell protein production: Biofilm vs
298 suspended growth. *Water Research*. 2020 181:115909.

299 6. George DM, Vincent AS, Mackey HR. An overview of anoxygenic phototrophic bacteria
300 and their applications in environmental biotechnology for sustainable Resource recovery.
301 *Biotechnology Reports*. 2020 28:e00563.

302 ●7. Delamare-Deboutteville J, Batstone DJ, Kawasaki M, Stegman S, Salini M, Tabrett S, et
303 al. Mixed culture purple phototrophic bacteria is an effective fishmeal replacement in
304 aquaculture. *Water Research X*. 2019 4:100031. *This paper shows the potential of*
305 *wastewater derived purple phototrophic bacteria (PPB) to substitute bulk fishmeal in*
306 *commercial aquaculture feed (Asian sea bass). This work is the first of its kind,*
307 *substituting large amounts of fishmeal and forms the basis for feed trials of this kind to*
308 *assess the PPB biomass value and potential.*

309 ●8. Alloul A, Wille M, Lucenti P, Bossier P, Van Stappen G, Vlaeminck SE. Purple bacteria
310 as added-value protein ingredient in shrimp feed: Penaeus vannamei growth
311 performance, and tolerance against Vibrio and ammonia stress. *Aquaculture*. 2021
312 530:735788. *This paper describes the addition of PPB biomass to shrimp feed and its*
313 *effectiveness. This is presents an outlook into the potential value of the PPB biomass as*
314 *single cell protein.*

315 9. Mohsenpour SF, Hennige S, Willoughby N, Adeloye A, Gutierrez T. Integrating micro-
316 algae into wastewater treatment: A review. *Science of The Total Environment*. 2021
317 752:142168.

318 10. Suparmaniam U, Lam MK, Uemura Y, Lim JW, Lee KT, Shuit SH. Insights into the
319 microalgae cultivation technology and harvesting process for biofuel production: A
320 review. *Renewable and Sustainable Energy Reviews*. 2019 115:109361.

321 11. Okoro V, Azimov U, Munoz J, Hernandez HH, Phan AN. Microalgae cultivation and
322 harvesting: Growth performance and use of flocculants - A review. *Renewable and*
323 *Sustainable Energy Reviews*. 2019 115:109364.

324 12. Alam MA, Xu J-L, Wang Z. Microalgae biotechnology for food, health and high value
325 products: Springer; 2020.

- 326 ●13. Umesh M, Santhosh AS. A Strategic Review on Use of Polyhydroxyalkanoates as an
327 Immunostimulant in Aquaculture. *Applied Food Biotechnology*. 2020 8:1-18. *This*
328 *review summarises various application studies of polyhydroxyalkanoates as aquafeed*
329 *additives and highlights their promising roles as eco-friendly alternatives for*
330 *commercial antibiotics with strong immunomodulatory effects in fish and shrimps. The*
331 *study reviews the use of polyhydroxyalkanoates and their copolymers as alternatives to*
332 *antibiotics in aquaculture and describes the scope of development of*
333 *polyhydroxyalkanoates as feed component against common pathogens in aquaculture.*
- 334 ●14. Nakano T, Wiegertjes G. Properties of carotenoids in fish fitness: a review. *Marine*
335 *Drugs*. 2020 18:568. *This paper reviews the use of ecologically friendly functional*
336 *carotenoids, particularly astaxanthin, as chemotherapeutic supplements in aquaculture*
337 *and its potential to improve the antioxidative state and immune system, increased disease*
338 *resistance, growth performance, survival, and improved egg quality in farmed fish*
339 *without exhibiting any cytotoxicity or side effects.*
- 340 ●15. Naylor RL, Hardy RW, Buschmann AH, Bush SR, Cao L, Klinger DH, et al. A 20-year
341 retrospective review of global aquaculture. *Nature*. 2021 593:E12-E. *This paper reviews*
342 *the developments in global aquaculture from 1997 to 2017, incorporating all industry*
343 *sub-sectors and highlighting the integration of aquaculture in the global food system. It*
344 *describes trends in feed efficiency and fish nutrition, fish-in–fish-out ratio, the*
345 *dependence on marine ingredients and reliance on terrestrial ingredients.*
- 346 16. Tacon AGJ. Trends in Global Aquaculture and Aquafeed Production: 2000-2017. *Rev*
347 *Fish Sci Aquac*. 2020 28:43-56.
- 348 17. Krogdahl A, Penn M, Thorsen J, Refstie S, Bakke AM. Important antinutrients in plant
349 feedstuffs for aquaculture: an update on recent findings regarding responses in
350 salmonids. *Aquaculture Research*. 2010 41:333-44.
- 351 18. Saejung C, Ampornpat W. Production and nutritional performance of carotenoid-
352 producing photosynthetic bacterium *Rhodospseudomonas faecalis* PA2 grown in
353 domestic wastewater intended for animal feed production. *Waste and Biomass*
354 *Valorization*. 2019 10:299-310.
- 355 19. Candebat CL, Booth MA, Pirozzi I. The sulfur amino acid requirements of juvenile
356 Yellowtail Kingfish (*Seriola lalandi*). *Aquaculture*. 2021 534:736234.
- 357 20. Jones SW, Karpol A, Friedman S, Maru BT, Tracy BP. Recent advances in single cell
358 protein use as a feed ingredient in aquaculture. *Current Opinion in Biotechnology*. 2020
359 61:189-97.
- 360 21. Chowdhury AJK, Zakaria NH, Abidin ZAZ. Phototrophic Purple Bacteria as Feed
361 Supplement on the Growth, Feed Utilization and Body Compositions of Malaysian
362 Mahseer, *Tor tambroides* Juveniles. *Sains Malaysiana*. 2016 45:135-40.
- 363 22. Loo PL, Chong VC, Ibrahim S, Sabaratnam V. Manipulating culture conditions and feed
364 quality to increase the survival of larval marble goby *Oxyeleotris marmorata*. *North*
365 *American Journal of Aquaculture*. 2015 77:149-59.
- 366 23. Chumpol S, Kantachote D, Rattanachuay P, Torpee S, Nitoda T, Kanzaki H.
367 Optimization of culture conditions for production of antivibrio compounds from
368 probiotic purple nonsulfur bacteria against acute hepatopancreatic necrosis disease-
369 causing *Vibrio parahaemolyticus* and *Vibrio* spp. *Aquaculture*. 2019 505:72-83.
- 370 24. LaTurner ZW, Bennett GN, San K-Y, Stadler LB. Single cell protein production from
371 food waste using purple non-sulfur bacteria shows economically viable protein products
372 have higher environmental impacts. *Journal of Cleaner Production*. 2020 276:123114.
- 373 25. Calvo NS, Reynoso CM, Resnik S, Cortés-Jacinto E, Collins P. Thermal stability of
374 astaxanthin in oils for its use in fish food technology. *Animal Feed Science and*
375 *Technology*. 2020 270:114668.

- 376 26. Pereira da Costa D, Campos Miranda-Filho K. The use of carotenoid pigments as food
377 additives for aquatic organisms and their functional roles. *Reviews in Aquaculture*. 2020
378 12:1567-78.
- 379 27. Sandmann G. Diversity and origin of carotenoid biosynthesis: its history of coevolution
380 towards plant photosynthesis. *New Phytologist*. 2021 n/a.
- 381 28. García-Chavarría M, Lara-Flores M. The use of carotenoid in aquaculture. *Research*
382 *Journal of Fisheries and Hydrobiology*. 2013 8:38-49.
- 383 29. Glencross BD, Huyben D, Schrama JW. The application of single-cell ingredients in
384 aquaculture feeds—a review. *Fishes*. 2020 5:22.
- 385 30. Lopez-Romero J, Salgado-Manjarrez E, Torres L, Garcia-Peña EI. Enhanced carotenoid
386 production by *Rhodospseudomonas palustris* ATCC 17001 under low light conditions.
387 *Journal of Biotechnology*. 2020 323:159-65.
- 388 31. Wernli D, Jorgensen PS, Parmley EJ, Troell M, Majowicz S, Harbarth S, et al. Evidence
389 for action: a One Health learning platform on interventions to tackle antimicrobial
390 resistance. *Lancet Infect Dis*. 2020 20:E307-E11.
- 391 32. Lulijwa R, Rupia EJ, Alfaro AC. Antibiotic use in aquaculture, policies and regulation,
392 health and environmental risks: a review of the top 15 major producers. *Rev Aquacult*.
393 2020 12:640-63.
- 394 33. Kalia VC, Singh Patel SK, Shanmugam R, Lee J-K. Polyhydroxyalkanoates: Trends and
395 advances toward biotechnological applications. *Bioresource Technology*. 2021
396 326:124737.
- 397 34. Kiran GS, Priyadharshini S, Sajayan A, Ravindran A, Priyadharshini GB, Ramesh U, et
398 al. Dietary administration of gelatinised polyhydroxybutyrate to *Penaeus vannamei*
399 improved growth performance and enhanced immune response against *Vibrio*
400 *parahaemolyticus*. *Aquaculture*. 2020 517:734773.
- 401 35. Wang X, Jiang XR, Wu F, Ma Y, Che X, Chen X, et al. Microbial poly- 3-
402 hydroxybutyrate (PHB) as a feed additive for fishes and piglets. *Biotechnology journal*.
403 2019 14:1900132.
- 404 36. Tran NT, Li Z, Wang S, Zheng H, Aweya JJ, Wen X, et al. Progress and perspectives of
405 short-chain fatty acids in aquaculture. *Reviews in Aquaculture*. 2020 12:283-98.
- 406 37. Fradinho J, Allegue LD, Ventura M, Melero JA, Reis MAM, Puyol D. Up-scale
407 challenges on biopolymer production from waste streams by Purple Phototrophic
408 Bacteria mixed cultures: A critical review. *Bioresource Technology*. 2021 327:124820.
- 409 38. Luongo V, Ghimire A, Frunzo L, Fabbicino M, d'Antonio G, Pirozzi F, et al.
410 Photofermentative production of hydrogen and poly- β -hydroxybutyrate from dark
411 fermentation products. *Bioresource Technology*. 2017 228:171-5.
- 412 39. Gowda V, Shivakumar S. Novel Biocontrol Agents: Short Chain Fatty Acids and More
413 Recently, Polyhydroxyalkanoates. *Biotechnological Applications of*
414 *Polyhydroxyalkanoates*: Springer; 2019, p. 323-45.
- 415 40. Tamis J, Lužkov K, Jiang Y, Loosdrecht MCMv, Kleerebezem R. Enrichment of
416 *Plasticicumulans acidivorans* at pilot-scale for PHA production on industrial wastewater.
417 *Journal of Biotechnology*. 2014 192:161-9.
- 418 41. Kumar BR, Mathimani T, Sudhakar MP, Rajendran K, Nizami A-S, Brindhadevi K, et
419 al. A state of the art review on the cultivation of algae for energy and other valuable
420 products: Application, challenges, and opportunities. *Renewable and Sustainable Energy*
421 *Reviews*. 2021 138:110649.
- 422 42. Moshood TD, Nawanir G, Mahmud F. Microalgae biofuels production: A systematic
423 review on socioeconomic prospects of microalgae biofuels and policy implications.
424 *Environmental Challenges*. 2021 5:100207.
- 425 43. Spiller M, Muys M, Papini G, Sakarika M, Buyle M, Vlaeminck SE. Environmental

- 426 impact of microbial protein from potato wastewater as feed ingredient: Comparative
427 consequential life cycle assessment of three production systems and soybean meal. *Water*
428 *Research*. 2020 171:115406.
- 429 44. Alloul A, Cerruti M, Adamczyk D, Weissbrodt DG, Vlaeminck SE. Operational
430 Strategies to Selectively Produce Purple Bacteria for Microbial Protein in Raceway
431 Reactors. *Environmental Science & Technology*. 2021.
- 432 45. Sepúlveda-Muñoz CA, Ángeles R, de Godos I, Muñoz R. Comparative evaluation of
433 continuous piggery wastewater treatment in open and closed purple phototrophic
434 bacteria-based photobioreactors. *Journal of Water Process Engineering*. 2020 38:101608.
- 435 46. Alloul A, Wuyts S, Lebeer S, Vlaeminck SE. Volatile fatty acids impacting phototrophic
436 growth kinetics of purple bacteria: Paving the way for protein production on fermented
437 wastewater. *Water Research*. 2019 152:138-47.
- 438 47. García D, de Godos I, Domínguez C, Turiel S, Bolado S, Muñoz R. A systematic
439 comparison of the potential of microalgae-bacteria and purple phototrophic bacteria
440 consortia for the treatment of piggery wastewater. *Bioresource Technology*. 2019
441 276:18-27.
- 442 48. López-Serna R, García D, Bolado S, Jiménez JJ, Lai FY, Golovko O, et al.
443 Photobioreactors based on microalgae-bacteria and purple phototrophic bacteria
444 consortia: A promising technology to reduce the load of veterinary drugs from piggery
445 wastewater. *Science of the Total Environment*. 2019 692:259-66.
- 446 49. Makhneva ZK, Ashikhmin AA, Bolshakov MA, Moskalenko AA. Quenchers Protect
447 BChl850 from Action of Singlet Oxygen in the Membranes of a Sulfur Photosynthetic
448 Bacterium *Allochromatium vinosum* Strain MSU. *Microbiology*. 2019 88:79-86.
- 449 50. Capson-Tojo G, Lin S, Batstone DJ, Hülsen T. Purple phototrophic bacteria are
450 outcompeted by aerobic heterotrophs in the presence of oxygen. *Water Research*. 2021
451 194:116941.
- 452 51. Hülsen T, Stegman S, Batstone DJ, Capson-Tojo G. Naturally illuminated
453 photobioreactors for resource recovery from piggery and chicken-processing
454 wastewaters utilising purple phototrophic bacteria. *Water Research in Submission*. 2021.
- 455 ●52. Alloul A, Cerruti M, Adamczyk D, Weissbrodt DG, Vlaeminck SE. Operational
456 Strategies to Selectively Produce Purple Bacteria for Microbial Protein in Raceway
457 Reactors. *Environmental Science & Technology*. 2021 55:8278-86. *This is among the*
458 *first paper describing the operational strategies to cultivate PPB in open raceway*
459 *ponds*.
- 460 ●53. Stegman S, Batstone DJ, Rozendal R, Jensen PD, Hülsen T. Purple phototrophic bacteria
461 granules under high and low upflow velocities. *Water Research*. 2021 190:116760. *This*
462 *paper is the first describing the bbtgarnulation of PPB biomass in an engineered system*
463 *which might open the way for direct feed applications e.g. in aquaculture*.
- 464 54. Cerruti M, Stevens B, Ebrahimi S, Alloul A, Vlaeminck SE, Weissbrodt DG. Enrichment
465 and aggregation of purple non-sulfur bacteria in a mixed-culture sequencing-batch
466 photobioreactor for biological nutrient removal from wastewater. *Frontiers in*
467 *bioengineering and biotechnology*. 2020 8:1432.
- 468

*Declaration of Interest Statement

none