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Abstract

Purple non-sulfur bacteria (PNSB) possess significant potential for bioresource recovery from wastewater. Effective operational tools are needed to boost productivity and direct the PNSB biomass towards abundant value-added substances (e.g., protein and co-enzyme Q10, CoQ10). This study aimed to investigate the impact of light, oxygen and volatile fatty acids (VFAs) on PNSB growth (i.e., *Rhodobacter sphaeroides*) and productivity of protein and CoQ10. Overall, the biomass yields and specific growth rates of PNSB were in the ranges of 0.57-1.08 g biomass g^{-1} $COD_{removed}$ and 0.48-0.71 d^{-1}, respectively. VFAs did not influence the biomass yield, yet acetate and VFA mixtures enhanced the specific growth rate with a factor of 1.2-1.5 compared to propionate and butyrate. The most PNSB biomass (1.08 g biomass g^{-1} $COD_{removed}$ and 0.71 d^{-1}) and the highest biomass quality (protein content of 609 mg g^{-1} dry cell weight (DCW) and CoQ10 content of 13.21 mg g^{-1} DCW) were obtained in the presence of VFA mixtures under natural light and microaerobic (low light alternated with darkness; dissolved oxygen (DO) between 0.5-1 mg L^{-1}) conditions (vs. light anaerobic and dark aerobic cultivations). Further investigation on VFAs dynamics revealed that acetate was most rapidly consumed by PNSB in the individual VFA feeding (specific uptake rate of 0.76 g COD g^{-1} DCW d^{-1}), while acetate as a co-substrate in the mixed VFAs feeding might accelerate the consumption of propionate and butyrate through providing additional cell metabolism precursor. Enzymes activities of succinate dehydrogenase and fructose-1,6-bisphosphatase as well as the concentration of photo pigments confirmed that light, oxygen and VFAs regulated the key enzymes in the energy

metabolism and biomass synthesis to boost PNSB growth. These results provide a promising prospect for utilization of fermented waste stream for the harvest of PNSB biomass, protein and CoQ10.

Keywords: purple non-sulfur bacteria; volatile fatty acids; light and oxygen; enzyme activity; protein; CoQ10

1. Introduction

Activated sludge process generated large amount of excess sludge, entailing disposal cost that accounted for 50% of the total operational cost (Dignac et al., 2000). Extensive studies were attempting to reduce activated sludge production, whilst a paradigm shift that recovered resource from wastewater was ongoing (Yadav et al., 2021). Despite chemo- and photoheterotrophic microorganisms were able to upgrade waste streams into value-added products, there exists a technological demand to optimize the efficiency in a cost-wise mode.

Purple non-sulfur bacteria (PNSB) may open a new route to simultaneously minimize waste and generate value-added substances (Alloul et al., 2021). PNSB are competent photoheterotrophs with the capabilities of photoautotrophy and dark chemotrophy (aerobic/anaerobic), which can efficiently uptake various organics as carbon sources for growth (George et al., 2020). PNSB attained a higher yield (1 g biomass g^{-1} $COD_{removed}$) than aerobic heterotrophic bacteria (0.44-0.55 g biomass g^{-1} $COD_{removed}$) (Puyol et al., 2017; Saejung and Thammaratana, 2016) and grew faster with specific growth rates of 0.6-3.7 d^{-1} than microalgae (0.6−1.1 d^{-1}) (Coppens et al., 2016). PNSB have been used to treat a variety types of non-toxic wastewater that is rich

in chemical oxygen demand (COD), such as starch, livestock and domestic sewage (Ponsano et al., 2008; Hülsen et al., 2016).

PNSB biomass is rich in polyhydroxyalkanoate (PHA), co-enzyme Q10 (CoQ10), carotenoid, bacteriochlorophyll, 5-ALA, protein, et cetera, which can be used to produce animal feed, probiotics, plastics, esters, et cetera (Cao et al., 2019). CoQ10, a naturally occurring fat-soluble quinone compound, has the functions of improving energy, boosting the immune system, and serving as a free radical scavenger (Tian et al., 2010). In the plasma membrane of prokaryotes, CoQ10 is an vital constituent of the electron transport system, serving as an acceptor/donor for electron transfer between complexes I/II and complex III (Park et al., 2005). The CoQ10 content in PNSB (6.34-12.96 mg g^{-1} DCW) exceeds that in *Escherichia coli* (1.3-3.8 mg g^{-1} DCW) and in animal tissues (0.02-0.08 mg g^{-1} DCW) (Ellis et al., 2012; Lamperti et al., 2003; Zhi et al., 2020). The microbial protein has been regarded as a dietary protein source for feed or food (Madukasi et al., 2010). Previous studies have reported that the pure or mixed cultures of PNSB yielded crude protein content of 40-80% of dry cell weight, which was higher than that in fungi (30-45%), aerobic heterotrophs (38-60%), microalgae (40-70%), et cetera (Honda et al., 2006; Alloul et al., 2021b).

Value-added products are unlikely produced at a consistent quality if the carbon source in wastewater is of diverse complexity. As a result, previous work proposed a three-step approach for converting COD into commodities, comprising capture of COD from sewage as sludge, generation of volatile fatty acids (VFAs) through anaerobic fermentation and production of marketable products using VFAs as the carbon source

(Alloul et al., 2018; Lee et al., 2014). VFAs consisting of directly assimilable small molecule organic acid, are more readily utilized by microorganisms and serve to be the desirable carbon source for PNSB growth to produce commodities (Chen et al., 2020).

PNSB possess two energy metabolisms that are photophosphorylation and oxidative phosphorylation and thus survive under light anaerobic, dark aerobic and facultative conditions (Imhoff and Bias-lmhoff, 2006). In PNSB-based sewage treatment, oxygen and light are two key parameters influencing not only the PNSB metabolic pathway but also pollutants removal and biomass growth (Meng et al., 2017). Biomass concentration of PNSB under oxic conditions was about 1.5 times higher than that under light anaerobic conditions and the involvement of oxygen enhanced COD removal efficiency from 80% to 96% (Lu et al., 2018). Various light and oxygen conditions might also have impacts on the accumulation of value-added products from PNSB, such as protein and CoQ10. However, the relevant knowledge is still scarce and the intrinsic link among light, oxygen, VFAs and PNSB is yet to be elucidated.

The purpose of this study was to clarify the effect of VFAs, light and oxygen on PNSB growth and value-added products. To achieve this, *Rhodobacter sphaeroides* (*Rb. sphaeroides*) were cultivated on various VFAs (acetate, propionate, butyrate and mixture of the first three) under different light and oxygen conditions (light anaerobic, natural light microaerobic and dark aerobic). Their combined effect on the biomass yield, the specific growth rate and the content of protein and CoQ10 was explored. The underlying mechanisms were explained through analyzing the results of the VFAs conversion, photo pigments accumulation and key enzymes activities.

2. Materials and methods

2.1 Microorganism and cultivation

A strain of PNSB used in this work was *Rb. sphaeroides*, which was isolated from a local pond in Wuhan, China (Yu et al., 2021). The purified species was pre-cultivated to maintain the microbial activity using medium adapted from Imhoff (Imhoff, 2006) in a thermostatic incubator (Tianjin Taisite Instrument Co., China) at 30 ± 1 °C with 3000-3500 lux provided by white LED lamps (7W, Philips, China). The features of *Rb. sphaeroides* and specific methods of cultivation are detailed in our previous study (Yu et al., 2021).

A VFA-based Imhoff medium was used for PNSB growth and value-added substances production (Alloul et al., 2019). Individual and combined VFAs (Sinopharm, Shanghai) containing equal amount of carbon were tested for the growth of *Rb. sphaeroides* in duplicate (per liter): (i) 0.93 g acetate, (ii) 0.74 g propionate, (iii) 0.66 g butyrate and (iv) combined VFAs (0.24 g acetate, 0.20 g propionate and 0.18 g butyrate, 1/1/1 proportion based on carbon mass). 1 mL trace element and 1 mL vitamin solution were dosed into the modified Imhoff medium. The details of the cultivation medium are listed in Supplementary Information S1 and Table S1. The pH was adjusted at 7.0 prior to the inoculation. The characteristics of the prepared culture medium for *Rb. sphaeroides* were as follows: The level of inorganic carbon (IC) was 0.3 g L^{-1}. COD, total phosphorus (TP) and total nitrogen (TN) was around 4600 mg COD L^{-1}, 230 mg P L^{-1} and 270 mg N L^{-1}, respectively.

2.2 Batch experimental setup

The experiments were performed in Schott bottles with working volume of 400 mL. The prepared fermentation medium was pre-autoclaved at 121°C for 20 min prior to inoculation. The *Rb. sphaeroides* were inoculated into autoclaved modified Imhoff medium in an incubator at 30 ± 1 °C with an initial inoculation ratio of 10% (v/v). The agitation was restricted to the range of 130-150 rpm to prevent cell damage. Each test lasted for 6 days, after which PNSB tended to cease growing due to the limitation of growth substrate.

Three light and oxygen conditions were shown below:

Light anaerobic condition: Two 60 W incandescent lamps (Beineng Lighting Co., Ltd. China) were used to keep the illumination in the range of 3000-3500 lux. Sterile paraffin liquid (Sinopharm, Shanghai) was used to maintain a strict anaerobic environment inside the reactor and prevent miscellaneous bacteria.

Natural light microaerobic condition: 12-h light (light intensity: 500-1500 lux) was alternated with 12-h darkness to mimic natural light. Microaerobic condition was achieved by controlling DO levels within the range of 0.5-1.0 mg L^{-1}.

Dark aerobic condition: The bioreactor was covered with tinfoil to prevent from light transmission. DO concentration during aerobic cultivation was kept above 2.0 mg L^{-1}.

2.3 Analytical methods

The Schott bottle was sealed by a screw cap containing a silicone rubber septum. When taking samples during the experiment, the bottles were turned upside down and the inverted septum was inserted by a needle connected a syringe for collection of 8-

mL mixed liquor samples every 24 hours. 3-mL samples were centrifuged at 9000 rpm for 15 min with a centrifuge (Eppendorf centrifuge 5430, Germany) to acquire supernatant for COD, TN, TP and VFA analysis. COD, TP and TN were measured in accordance with APHA standard methods (Gilcreas, 1966). VFA analysis was performed after pre-filtration of supernatant with a 0.45 μm syringe filter (Jinlong, Tianjin). A 0.4 μL pretreated sample was added to gas chromatography (Lunan Ruihong SP- 7820, Shandong) equipped with a flame ionization detector (GC/FID). The pH, DO and light intensity were monitored by a pH tester (Mettler Toledo, China), a dissolved oxygen meter (Leici JPBJ-610L, China) and a Lux light meter (TES 1330A, Taiwan), respectively.

The remaining 5-mL samples were centrifuges to collected PNSB cells for analysis of protein, CoQ10 and key enzymatic activities. Protein determination were performed according to the modified Lowry methods (Hülsen et al., 2018). CoQ10 was extracted from *Rb. sphaeroides* using organic solvents (ethyl acetate: ethanol = 5:3 (v: v)), and the absorbance were tested by Craven method with an assay kit (Beijing Leagene Biotechnology Co.) at 620 nm. The detection limits of protein and CoQ10 are 9.5 mg g^{-1} DCW and 0.026 mg g^{-1} DCW, respectively. The key enzymatic activities in the PNSB metabolic pathways, including succinate dehydrogenase (SDH) and fructose-1,6-bisphosphatase (FBP), were determined spectrophotometrically by the kits (Beijing Solarbio Science& Technology Co., Lt) at the wavelength of 600 nm and 340 nm, respectively. Carotenoids and bacteriochlorophylls were determined by a spectrophotometer at the wavelength of 473 nm and 771 nm, respectively and the

content were calculated according to the formula (1) and (2) (Zhou et al., 2014):

Carotenoid content $=A_{473}\times 10000/(250\times L\times W)$ (1)

Bacteriochlorophyll content $=A_{771}\times 10000/(76\times L\times W)$ (2)

where A_{473} and A_{771} represent the optical density of extract at 473 nm and 771 nm, respectively; L represents the pathlength of the cuvette; and W is the sample's initial mass (g) divided by the ultimate volume (mL) of the extraction solution.

Note that as enzymes were easily inactivated, fresh samples had to be kept on ice during the measurement to guarantee maximum activity. Since the pigments and CoQ10 tended to decompose in light, the whole extraction process should be carried out under dark conditions. The remaining samples had to be stored at -80 °C.

To minimize the interference by the decrease of liquid volume and loss of the PNSB biomass, 50-mL bacteria suspension was taken at the end of each test on day 6 from the Schott bottle for determination of the biomass concentration. The bacteria suspension was diluted into different concentration gradients for measurement of the optical density at 660 nm (OD_{660}) by a spectrophotometer (UV-5500PC, Shanghai Metash Instruments Co., Ltd. China). The DCW was measured by gravimetric method in triplicate. 4-mL samples from centrifuge tube were centrifuged at 9000 rpm for 15 min. After discharging supernatant, the remaining cells were transferred to a drying oven at 75 °C. The biomass-optical density curve was obtained and the daily dry cell weight could be calculated from the linear correlation between OD_{660} and DCW. The attained DCW was used to calculate biomass yield (g biomass g^{-1} $COD_{removed}$) and specific growth rate (d^{-1}).

197 2.4 Statistical analysis

198 All analyses were performed in duplicate. The values in this work represented the
199 mean values of two parallel tests to ensure data accuracy. The error bars corresponded
200 to the standard deviations. The means ± standard deviation (Means ± SD) was used to
201 express the experimental results. SPSS Statistics package (version 11.5, SPSS Inc.,)
202 was further adopted for variance analysis (ANOVA). The significances of the values
203 were analyzed using Tukey's test. $P < 0.05$ was taken as the significant level of
204 difference.

205 **3. Results and discussion**

206 3.1 PNSB growth on individual and combined VFAs under varying light and oxygen
207 conditions

208 The biomass yields and specific growth rates of PNSB under different conditions
209 were shown in Figure 1. The biomass yields were between 0.57-1.08 g biomass g^{-1}
210 $COD_{removed}$. The individual and combined VFAs had no noticeable effect on the biomass
211 yield ($P > 0.05$), as observed for all light and oxygen conditions. Previous studies
212 reported that the yield of *Rb. sphaeroides* in the fermentation medium loaded with
213 VFAs was close to 1 g biomass g^{-1} $COD_{removed}$ (Puyol et al., 2017), which was
214 comparable to the values of this study. The average biomass yield in natural light
215 microaerobic cultivations was roughly 1.1 times of that in light anaerobic cultivations
216 and 1.7 times of that in dark aerobic cultivations. The maximum biomass yield
217 exceeding 1 g biomass g^{-1} $COD_{removed}$ under natural light microaerobic condition was
218 possibly due to photo-assimilation of a highly reduced electron donor (i.e., propionate

and butyrate) along with CO_2 to compensate for excess reducing power (Barber and Andersson, 1992). Similarly, Yang et al. (2018) observed that the optimal PNSB biomass of 470 mg VSS L^{-1} were acquired under natural light aerobic condition. The light assimilation that converting CO_2 to COD under light conditions and the increased COD oxidation to CO_2 under aerobic conditions might explain the above-mentioned discrepancy in terms of PNSB biomass yields (Nakajima et al., 1997).

Different from the observations on the biomass yield, VFAs exerted a pronounced effect on the specific growth rate of PNSB. In each light and oxygen condition, the change of the specific growth rate upon VFAs followed a similar trend. The specific growth rates in groups fed on acetate and VFA mixtures were 1.1-1.5 times higher than those fed on butyrate and propionate. Similar observation was made by Alloul et al. (2019) that the VFA mixtures increased the specific growth rate of PNSB by a factor of 1.1 to 2.5 times compared to the individual VFA. The variation of light and oxygen conditions led to alteration of specific growth rates as well. The average specific growth rate under natural light microaerobic condition was comparable to that under dark aerobic condition, but 1.4 times higher than that under light anaerobic condition. Our results are in line with literatures (Alloul et al., 2021), jointly demonstrating that aerobic condition was more conducive to rapid growth of PNSB than anaerobic condition. The maximum specific growth rate of 0.71 d^{-1} was attained in the groups fed on VFA mixtures under natural light microaerobic condition, resulting in a doubling time of ~1 d. Hence, it can be envisioned that *Rb. sphaeroides* could be dominant over slow-growing microbes in a real wastewater environment in condition that proper light,

241 oxygen and VFAs were provided to shorten its doubling time.

242 The specific growth rate is a function of substrate uptake rate, biomass yield and
243 maintenance rate (Rombouts et al., 2018). Higher specific growth rate can be achieved
244 by maximizing growth substrate utilization and biomass yield. Comparable yield in this
245 study denoted that PNSB had a similar synthesis efficiency of varying VFAs.
246 Consequently, the higher specific growth rates on acetate and mixed VFAs were
247 attributed to a higher COD uptake rate by PNSB. To further validate this, a statistical
248 analysis of the data was performed to investigate COD degradation kinetics (details in
249 Supplementary Information S2). Table S2 concluded the first-order rate constants k of
250 COD degradation with R^2 above 0.94 under different VFAs, light and oxygen
251 conditions. The higher uptake rate of PNSB growth substrate was demonstrated by the
252 higher COD removal of 79% and greater k value of 0.289 attained in the group fed on
253 acetate under natural light microaerobic condition (Figure S1 and Table S2). Pollutants
254 were adequately mineralized and utilized via respiration for the production of biomass
255 under aerobic conditions. In contrast, the electronic acceptor was the fermentation
256 product rather than oxygen under light anaerobic condition, resulting in incomplete
257 mineralization of the original organics (Lu et al., 2020). Natural light microaerobic
258 condition combined the advantages of both oxygen and light metabolisms, where partial
259 energy derives from chemical catabolism and part from light. Hence, PNSB grow faster
260 under natural light microaerobic condition.

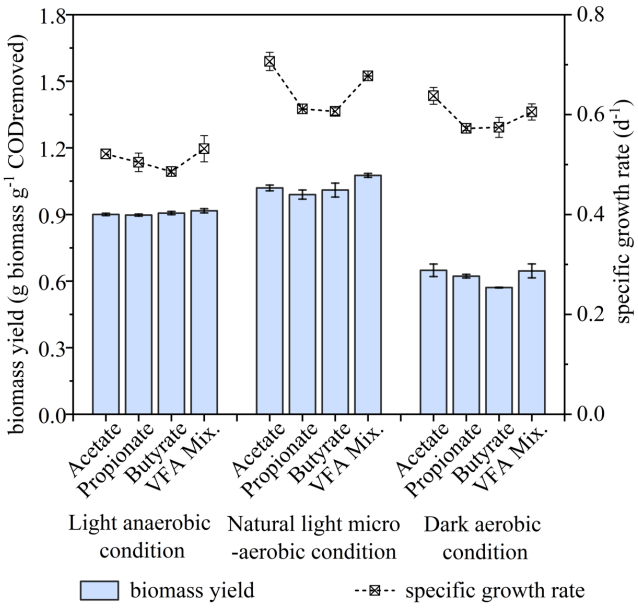

Figure 1. Biomass yield (left y-axis) and specific growth rate (right y-axis) on individual VFA and combined VFAs under varying light and oxygen conditions. Error bars show standard errors.

3.2 Value-added substances productivity on individual and combined VFAs under varying light and oxygen conditions

Figure 2 presented an overview of protein content in a histogram and a heat map under varying VFAs, light and oxygen conditions. The protein content under each light and oxygen condition was lowest when using butyrate as carbon source, averaging 484 mg g^{-1} DCW. Under all light and oxygen conditions, the protein content was enhanced by feeding acetate and VFA mixtures, while there was no substantial difference ($P < 0.05$) between the protein contents fed on the two. The enhancement was most notably under natural light microaerobic conditions, reaching 605 mg g^{-1} DCW for acetate and 609 mg g^{-1} DCW for VFA mixtures, both of which accounted for over 60% of the PNSB cells. Protein content in PNSB was also greatly affected by light and oxygen conditions ($P < 0.05$). The average protein content with feedings of all four VFAs conditions under dark aerobic condition (523 mg g^{-1} DCW) was remarkably higher than that under light

277 anaerobic condition (492 mg g^{-1} DCW), yet lower than that under natural light
278 microaerobic condition (568 mg g^{-1} DCW). From the heatmap of Figure 2b, it was
279 evident that PNSB grown on acetate or VFA mixtures under natural light microaerobic
280 condition were preferable for protein production. Our results were supported by
281 previous work (Lu et al., 2011), which investigated macromolecule protein removal by
282 *Rhodopseudomonas sphaeroides* under different light and oxygen conditions and
283 concluded that an elevated protein removal was obtained under aerobic condition rather
284 than light anaerobic condition.

285 The CoQ10 was also investigated as value-added product (Figure 3). The
286 variation of light, oxygen and VFAs conditions evidently affected the CoQ10
287 production by PNSB, with a P-value of 0.001 according to ANOVA analysis. Cells use
288 mRNA as a template to synthesize protein, while the microbial anabolic process of
289 CoQ10 includes the shikimate pathway and the synthesis of polyisoprene
290 pyrophosphate and quinone ring core (Clarke, 2000). Although the synthesis
291 mechanisms of protein and CoQ10 were distinct, a similar trend in their content
292 changing with VFAs, light and oxygen was observed. The CoQ10 content in this study
293 ranged from 7.59 to 13.21 mg g^{-1} DCW. PNSB grown on VFA mixtures achieved higher
294 average CoQ10 content than that on individual VFA. This discrepancy is likely to be
295 related to the cell metabolism efficiency, which would be further discussed in section
296 3.3 and 3.4. CoQ10 production (average values) under natural light microaerobic
297 condition were 1.4 and 1.2 times higher than that under light anaerobic condition and
298 dark aerobic condition, respectively. The highest CoQ10 content, 13.21 mg g^{-1} DCW,

was achieved in the group fed on VFA mixtures under natural light microaerobic condition (Figure 3b), which exceeded the reported range of CoQ10 content in PNSB (6.34-12.96 mg g^{-1} DCW) (Zhi et al., 2020). The comparatively high CoQ10 content is of great benefit due to its high market value (1100 US$ kg^{-1}) (Zhi et al., 2019). Generally, PNSB grown on acetate or VFA mixtures under natural light microaerobic condition could yield more protein and CoQ10.

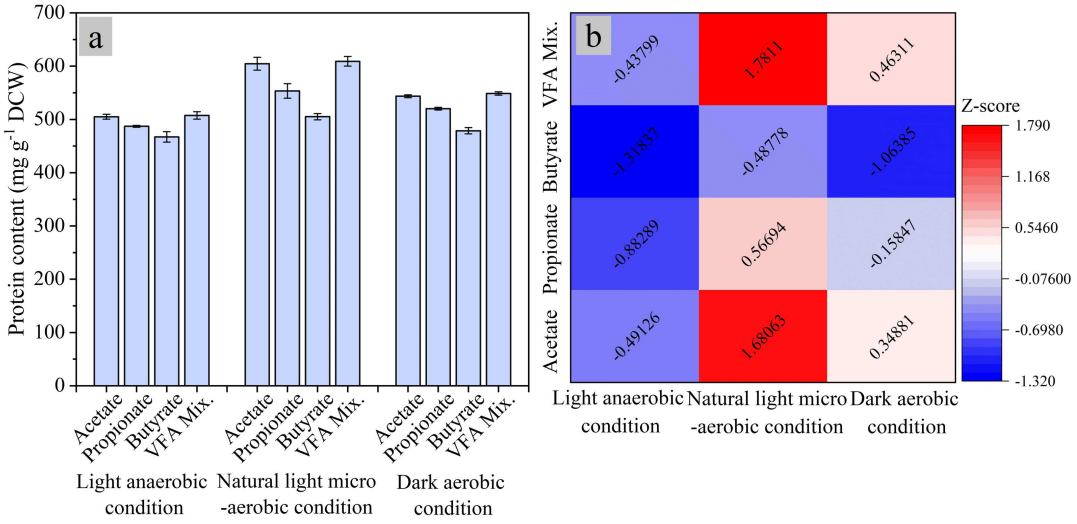

Figure 2. Overview of protein on individual and combined VFAs under varying light and oxygen conditions. Error bars show standard errors. (a) protein content in a histogram and (b) relative protein content in a heatmap. The value of protein content was standardized to make them in the same magnitude in the heat map. The greater Z-score represents the high level of relative protein content.

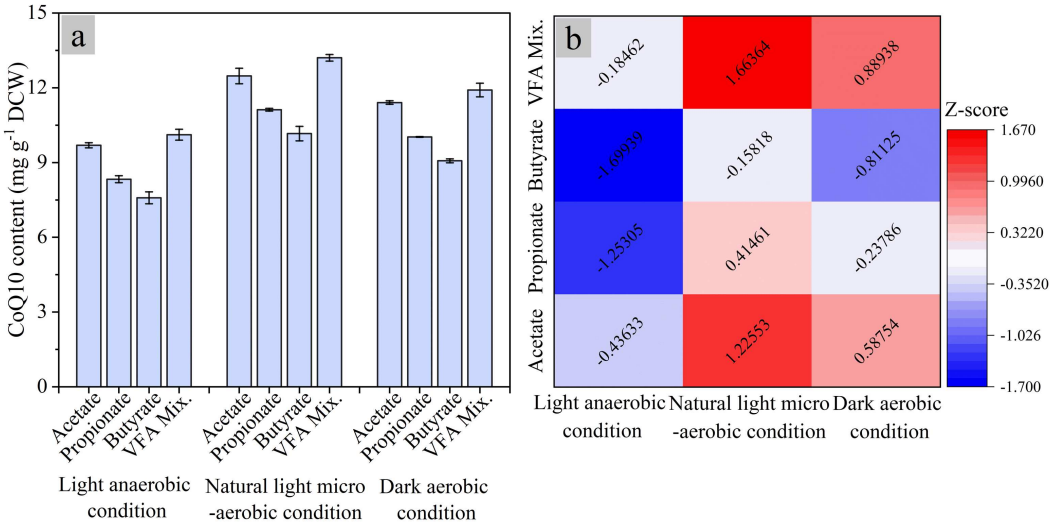

Figure 3. CoQ10 content on individual VFA and combined VFAs under varying light and oxygen conditions. Error bars show standard errors. (a) CoQ10 content in a histogram and (b) relative CoQ10 content in a heat map.

3.3 VFAs uptake profile under natural light microaerobic condition

The individual consumption profiles of acetate, propionate and butyrate by PNSB under natural light microaerobic condition were presented in Figure 4a. The initial concentrations (measured by GC) of acetate, propionate and butyrate were 1075, 1069 and 1067 mg COD L^{-1}, respectively, which slightly differed from theoretical values possibly attributing to measurement errors. Acetate was depleted within 3 days, while propionate and butyrate were slowly consumed with the residual concentrations of 224 and 288 mg COD L^{-1} on the 6^{th} day, respectively. The specific uptake rates of acetate, propionate and butyrate were 0.76, 0.27 and 0.21 g COD g^{-1} DCW d^{-1}, respectively. A similar observation was reported that the acetate uptake rate by PNSB was 1.5 and 1.8 times higher than that of propionate and butyrate, respectively (Lemos et al., 2006). In terms of PNSB metabolic pathways, acetate was directly converted to acetyl-CoA, a prominent precursor for cell metabolism, while the conversions of propionate and

butyrate to acetyl-CoA needed additional steps. As to propionate, it was transformed to propionyl-CoA, and portion of propionyl-CoA was decarboxylated to acetyl-CoA. Butyrate must undergo a higher number of conversions that oxidized to butyryl-CoA firstly, acetoacetyl-CoA secondly, then cleavage to acetyl-CoA (Choorit et al., 2011). This accounts for the slower consumption rate of propionate and butyrate compared to acetate. The quicker utilization of acetate also explained the higher specific growth rate of PNSB on acetate than propionate and butyrate.

The dynamic profiles and specific uptake rate of co-existing acetate, propionate and butyrate under natural light microaerobic condition were shown in Figure 4b. With the feeding of mixed VFAs, propionate was converted more quickly, followed by acetate and then butyrate. The specific uptake rates of propionate (0.75 g COD g^{-1} DCW d^{-1}) and butyrate (0.25 g COD g^{-1} DCW d^{-1}) with the feeding of mixed VFAs were 2.7 and 1.3 times higher than those fed on individual VFAs, respectively. In contrast, the specific uptake rate of acetate here (0.48 g COD g^{-1} DCW d^{-1}) was much lower than that fed on acetate individually (0.75 g COD g^{-1} DCW d^{-1}). This might result from the competitive relationship between the substrates (Wang et al., 2018). With feeding of mixed VFAs, acetate as a co-substrate could provide more acetyl-CoA for conversion of propionate and butyrate, thus promoting the metabolic efficiency of PNSB cells (Fradinho et al., 2014). The existence of co-substrates also induces quicker growth and faster carbon source removal. The higher biomass growth, protein and CoQ10 content obtained in the presence of individual acetate (Figure 1-3) might be attributed to the higher individual acetate uptake (Figure 4a). Similarly, the VFA mixtures as the optimal

substrate for PNSB-based resource recovery might be associated with the promoting effect of acetate as a co-substrate (Figure 4b). It should be noted that CO_2 fixation was considered as the dominant redox homeostasis mechanism in PNSB, where the more reduced VFA require more CO_2 supplement as the electron sink (Segura et al., 2021). Experiments have proved that 10 mM of propionate or 15 mM of butyrate as the sole carbon source would be completely assimilated in the presence of 3 mM bicarbonate, while VFA mixtures would largely reduce the bicarbonate requirement (Segura et al., 2021). The level of bicarbonate in the Imhoff medium (around 3.6 mM) of this study was adequate to maintain the redox balance in any investigated conditions.

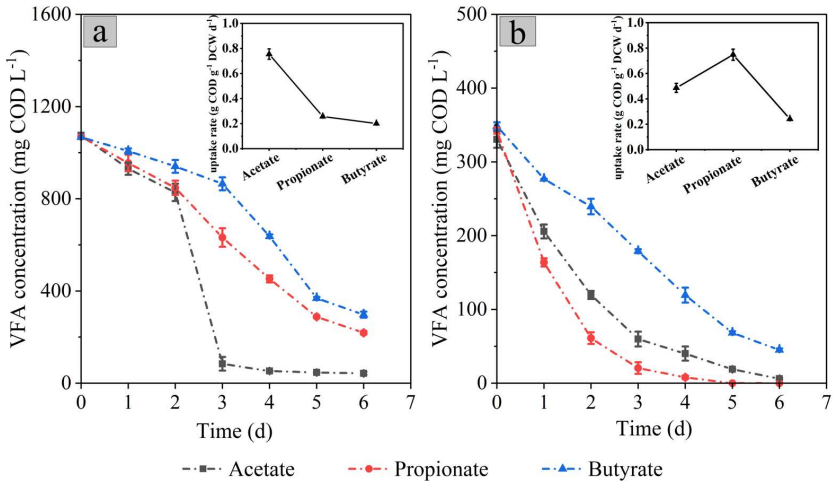

Figure 4. The consumption of individual VFA and a mixed VFAs under natural light microaerobic condition. (a) individual VFA consumption profile, (b) combined VFAs consumption profile. Error bars show standard errors.

3.4 Determination of key enzymes in metabolic pathways of PNSB

The activities of key enzymes in the energy metabolism and biomass synthesis pathways of PNSB were related to the accumulation of intracellular components in *Rb. sphaeroides* (Govindjee and Krogmann, 2005). To elucidate the mechanism, the impact

368 of light and oxygen conditions on key enzymes with acetate as the carbon source was
369 shown in Figure 5a, c & e, while enzyme response to varying VFAs as carbon sources
370 under natural light microaerobic condition was depicted in Figure 5b, d & f.

371 SDH was a crucial electron transporter of tricarboxylic acid cycle (TCA cycle) in
372 the respiratory energy metabolic pathway (Gutman et al., 1971). Compared to light
373 anaerobic condition, SDH activity under natural light microaerobic and dark aerobic
374 conditions increased by 62% and 54%, respectively (Figure 5a). The presence of DO
375 facilitated SDH activity, but higher DO would potentially inhibit the expression of SDH
376 subunits (Hancock et al., 2016). The higher SDH activity under natural light
377 microaerobic condition might also be attributed to light-dark cycles. It was reported
378 that exposure of *Rhodopseudomonas* to an appropriate light-dark cycle, as opposed to
379 consecutive illumination, enhanced biomass production (Zhi et al., 2019). The SDH
380 activity in groups fed on acetate and VFA mixtures were 1.2-1.4 times higher than those
381 fed on propionate and butyrate (Figure 5b). As described in Section 3.3, higher specific
382 uptake rate was achieved in the group fed on acetate, which accelerated electronic
383 delivery of oxidative phosphorylation process, thus resulting in higher SDH activity.

384 Photosynthetic electron transporter bacteriochlorophyll and carotenoid were key
385 enzymes in the photosynthetic energy metabolic pathway (Jones et al., 1990). The total
386 photo-pigment content under light anaerobic condition were 16.41 mg g^{-1} DCW
387 composing of ~38% carotenoid and ~62 % bacteriochlorophyll, which was 1.4 times
388 higher than that under dark aerobic condition (Figure 5c). This might be due to the fact
389 that photo-pigment synthesis ultimately ceased in the complete darkness (Koku et al.,

2002). Light was known to regulate the expression of photosynthetic genes (bchF, bchC, bchE, crtA) that controlled the synthesis of carotenoid and bacteriochlorophyll and suitable light and oxygen showed a facilitative effect on photo-pigment synthesis (Juri et al., 2013). Similar to the trend observed in PNSB growth (Figure 1) and value-added products (Figure 2&3), the groups fed on acetate and VFA mixtures achieved photo-pigment content of 15.53 and 15.86 mg g^{-1} DCW, respectively, which were higher than those fed on butyrate and propionate (Figure 5d). Photo pigments is intracellular substances of PNSB, positively correlated to PNSB biomass (Bauer, 2006). Therefore, acetate and mixed VFAs were more conducive to PNSB growth, resulting in increased photo pigments. Acetate was utilized more quickly than butyrate by PNSB for photo-pigment production, with 5% and 60% remaining on day 4, respectively (Figure 4a). Studies showed that acetate as a carbon source significantly increased the growth rate and cell yield of *Spirulina* and almost doubled the pigment content (Judd et al., 2015).

PNSB generate more reduced electron carriers than requirement for cell synthesis under photoheterotrophic conditions. To maintain redox homeostasis PNSB mostly depend on the Calvin cycle to oxidize these excess reduced equivalents (Bayon-Vicente et al., 2021; McCully et al., 2020). FBP was a key enzyme to supply NADPH and catalysed a rate-limiting step in Calvin cycle of phototrophic bacteria (Tang et al., 2011).

FBP activity in light anaerobic cultivation was 49% and 18% lower than that in natural light microaerobic and dark aerobic cultivation, respectively (Figure 5e). The highest activity of FBP, 1789.81 U g^{-1} DCW, was achieved under natural light microaerobic

conditions. The higher FBP activity of 1789.81 and 1837.89 U g^{-1} DCW were attained in groups fed on acetate and VFA mixtures, respectively (Figure 5f). In line with our findings, Peng and Shimizu (2003) evaluated the FBP activity discrepancies in groups fed on glucose, acetate and glycerol and higher FBP activity was observed when acetate served as the carbon source, indicating that VFAs were desirable growth substrates. It was also found that the specific growth rate was closely associated with the heterologous expression of bacterial FBP (Xu et al., 2014). As the reduced VFAs were supplied as the carbon source for PNSB growth in this study, a more active Calvin cycle was required (Segura et al., 2021). Higher functional enzyme activity led to higher biomass growth as well as protein and CoQ10 content (Gutman et al., 1971). Our results displayed a consistency between the responses of key enzymes and the changes of PNSB biomass and value-added products upon varying VFAs, light and oxygen conditions.

As shown in Figure S2 in supplementary information S3, under aerobic condition, electrons are transferred from NADH via CoQ or cytochrome C2 oxidase to the ultimate acceptor O_2 for driving ATP synthesis. Under light anaerobic condition, photo-phosphorylation is the main pathway for the synthesis of ATP. Catabolic pathways that generated more ATPs per unit substrate at similar anabolic efficiencies resulted in a higher yield (Rombouts et al., 2018). The highest SDH activity was obtained under natural light microaerobic condition (Figure 5a), where respiration was more active and oxidative phosphorylation occupies a leading role, producing amounts of ATPs for PNSB growth. The ATP was also required for the synthesis of intracellular protein and

CoQ10 (Zhou et al., 2014). As shown in Figure S2, energy for PNSB growth was mainly generated by the degradation of VFAs through the TCA cycle and electron transport chain. SDH connects the TCA cycle and respiratory chain. PNSB grown on acetate gained higher enzyme activity and thus resulted in more active TCA cycle, promoting the reaction of oxidized phosphorylation on the electron transfer chain to synthesize a large number of ATPs for biomass growth. The superiority of acetate over other individual VFAs was because that acetyl-CoA guided acetate directly to major metabolic pathways (Fradinho et al., 2014). Unlike SDH activity, the highest photo-pigment content was achieved under light anaerobic conditions. As presented in Figure S2, PNSB were able to utilize light to synthesize ATP. The presence of oxygen could destroy photosynthetic cytochromes, resulting in damage of the inner membrane of the photosynthetic system, which in turn weakened the energy metabolic pathway of photophosphorylation (Forti et al., 2006). Light intensity and photoperiod are essential factors affecting pigment production (Zhou et al., 2014). The content of photo pigments determined the energy harvested by PNSB, which influenced biomass growth. According to previous work (Schagerl and Müller, 2006), carotenoid and bacteriochlorophyll served as photo-harvesters under low light condition and became photo-protector when exposed to intense light. Consistent with our results, it has been reported that exposure of *Rhodopseudomonas* to proper light/dark cycles enhanced photo-pigment production and PNSB growth (Zhi et al., 2019). Furthermore, the similar trends of FBP activities, VFA consumption and protein content upon varying VFAs, light and oxygen in this study confirmed that oxidation of excess reductant not used in

PNSB biomass synthesis was coupled to CO_2 fixation in Calvin cycle (McCully et al., 2020). To sum up, light, oxygen and VFAs are essential factors affecting the production of biomass, protein and CoQ10 by *Rb. sphaeroides* via regulation of the key enzymes in the energy metabolism and biomass synthesis.

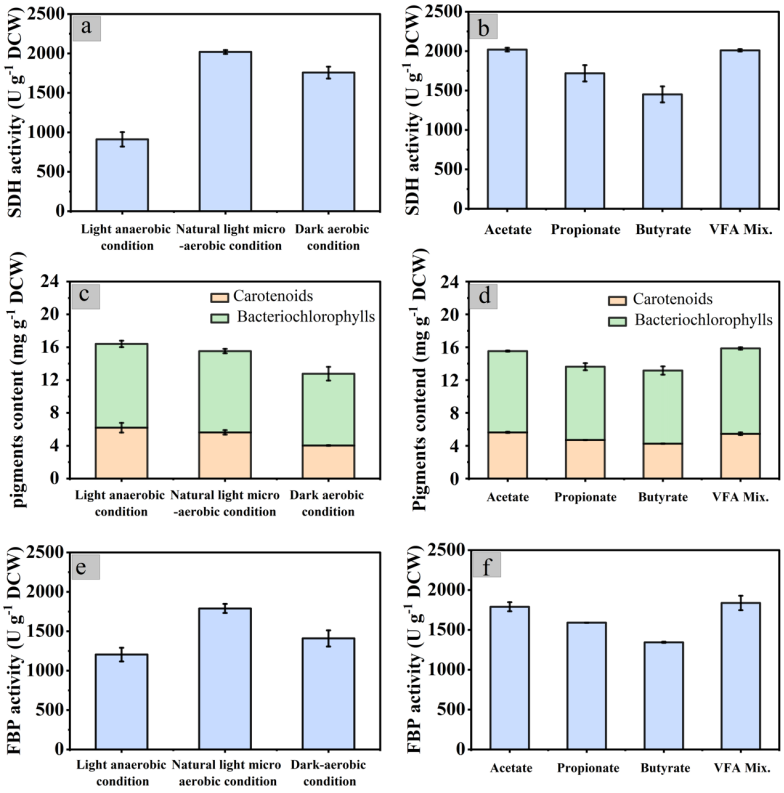

Figure 5. The effect of varying VFAs, light and oxygen conditions on the activities of succinate dehydrogenase (SDH), photo pigments and Fructose-1,6-bisphosphatase (FBP). The effect of light and oxygen on SDH activity (a), carotenoid and bacteriochlorophyll activity (c), FBP activity (e), with acetate as the carbon source and the effect of varying VFAs on SDH activity (b), carotenoid and bacteriochlorophyll activity (d), FBP activity (f) under natural light microaerobic condition. Error bars show standard errors.

4. Conclusions

This study investigated the effect of VFA, light and oxygen on PNSB growth with the main conclusions listed below:

- The highest biomass yield and specific growth rate was 1.08 g biomass g^{-1} $COD_{removed}$ with feeding of VFA mixtures and 0.71 d^{-1} with feeding of acetate, respectively under natural light microaerobic condition.
- Natural light microaerobic condition and acetate/ VFA mixtures as carbon source were also more conducive to protein (up to 609 mg g^{-1} DCW) and CoQ10 accumulation (13.21 mg g^{-1} DCW).
- Individual acetate was consumed by PNSB most quickly with a specific uptake rate of 0.76 g COD g^{-1} DCW d^{-1}, while the co-substrate acetate enhanced uptake rates of propionate and butyrate.
- The activity profiles of SDH, FBP, carotenoid and bacteriochlorophyll were in line with those of PNSB biomass, confirming that light, oxygen and VFAs altered PNSB growth via regulation of the key enzymes in the energy metabolism and biomass synthesis.

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Reference

Alloul, A., Cerruti, M., Adamczyk, D., Weissbrodt, D.G., Vlaeminck, S.E., 2021a. Operational strategies to selectively produce purple bacteria for microbial protein in raceway reactors. Environ. Sci. Technol. 55, 8278–8286.

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, 6729–6742.

Alloul, A., Muys, M., Hertoghs, N., Kerckhof, F.M., Vlaeminck, S.E., 2021b. Cocultivating aerobic heterotrophs and purple bacteria for microbial protein in sequential photo- and chemotrophic reactors. Bioresour. Technol. 319, 124192.

Alloul, A., Wuyts, S., Lebeer, S., Vlaeminck, S.E., 2019. Volatile fatty acids impacting phototrophic growth kinetics of purple bacteria: Paving the way for protein production on fermented wastewater. Water Res. 152, 138–147.

Barber, J., Andersson, B., 1992. Too much of a good thing: light can be bad for photosynthesis. Trends Biochem. Sci. 17, 61–66.

Bauer, C.E., 2006. Regulation of Photosynthesis Gene Expression. Anoxygenic Photosynth. Bact. 1221–1234.

Bayon-Vicente, G., Marchand, E., Ducrotois, J., Dufrasne, F.E., Hallez, R., Wattiez, R.,

513 Leroy, B., 2021. Analysis of the Involvement of the Isoleucine Biosynthesis
514 Pathway in Photoheterotrophic Metabolism of Rhodospirillum rubrum. Front.
515 Microbiol. 12.
516 Cao, K., Zhi, R., Zhang, G., 2019. Bioresource Technology Photosynthetic bacteria
517 wastewater treatment with the production of value- added products : A review.
518 Bioresour. Technol. 122648.
519 Chen, J., Wei, J., Ma, C., Yang, Z., Li, Z., Yang, X., Wang, M., Zhang, H., Hu, J.,
520 Zhang, C., 2020. Photosynthetic bacteria-based technology is a potential
521 alternative to meet sustainable wastewater treatment requirement? Environ. Int.
522 137, 105417.
523 Choorit, W., Saikeur, A., Chodok, P., Prasertsan, P., Kantachote, D., 2011. Production
524 of biomass and extracellular 5-aminolevulinic acid by Rhodopseudomonas
525 palustris KG31 under light and dark conditions using volatile fatty acid. J. Biosci.
526 Bioeng. 111, 658–664.
527 Clarke, C.E., 2000. New advances in coenzyme Q biosynthesis 134–147.
528 Coppens, J., Lindeboom, R., Muys, M., Coessens, W., Alloul, A., Meerbergen, K.,
529 Lievens, B., Clauwaert, P., Boon, N., Vlaeminck, S.E., 2016. Nitrification and
530 microalgae cultivation for two-stage biological nutrient valorization from source
531 separated urine. Bioresour. Technol. 211, 41–50.
532 Dignac, M.F., Ginestet, P., Rybacki, D., Bruchet, A., Urbain, V., Scribe, P., 2000. Fate
533 of wastewater organic pollution during activated sludge treatment: Nature of
534 residual organic matter. Water Res. 34, 4185–4194.

Ellis, J.T., Sims, R.C., Miller, C.D., 2012. Monitoring microbial diversity of bioreactors using metagenomic approaches, Subcellular Biochemistry.

Forti, G., Agostiano, A., Barbato, R., Bassi, R., Brugnoli, E., Finazzi, G., Garlaschi, F.M., Jennings, R.C., Melandri, B.A., Trotta, M., Venturoli, G., Zanetti, G., Zannoni, D., Zucchelli, G., 2006. Photosynthesis research in Italy: A review. Photosynth. Res. 88, 211–240.

Fradinho, J.C., Oehmen, A., Reis, M.A.M., 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.

George, D.M., Vincent, A.S., Mackey, H.R., 2020. An overview of anoxygenic phototrophic bacteria and their applications in environmental biotechnology for sustainable Resource recovery. Biotechnol. Reports 28, e00563.

Gilcreas, F.W., 1966. Standard methods for the examination of water and waste water. Am. J. Public Health Nations. Health 56, 387–388.

Govindjee, Krogmann, D., 2005. Discoveries in oxygenic photosynthesis (1727 – 2003): a perspective 63–105.

Gutman, M., Kearney, E.B., Singert, T.P., 1971. Regulation of Succinate Dehydrogenase Activity by Reduced Coenzyme Q10 10, 2726–2733.

Hancock, C.N., Liu, W., Alvord, W.G., Phang, J.M., 2016. Co-regulation of mitochondrial respiration by proline dehydrogenase/oxidase and succinate. Amino Acids 48, 859–872.

557 Honda, R., Fukushi, K., Yamamoto, K., 2006. Optimization of wastewater feeding for
558 single-cell protein production in an anaerobic wastewater treatment process
559 utilizing purple non-sulfur bacteria in mixed culture condition 125, 565–573.
560 Hülsen, T., Barry, E.M., Lu, Y., Puyol, D., Keller, J., Batstone, D.J., 2016. Domestic
561 wastewater treatment with purple phototrophic bacteria using a novel continuous
562 photo anaerobic membrane bioreactor. Water Res. 100, 486–495.
563 Hülsen, T., Hsieh, K., Lu, Y., Tait, S., Batstone, D.J., 2018. Simultaneous treatment
564 and single cell protein production from agri-industrial wastewaters using purple
565 phototrophic bacteria or microalgae – A comparison. Bioresour. Technol. 254,
566 214–223.
567 Imhoff, J.F., Bias-lmhoff, U., 2006. Lipids, Quinones and Fatty Acids of Anoxygenic
568 Phototrophic Bacteria. Anoxygenic Photosynth. Bact. 179–205.
569 Jones, M.R., McEwan, A.G., Jackson, J.B., 1990. The role of c-type cytochromes in the
570 photosynthetic electron transport pathway of Rhodobacter capsulatus. BBA -
571 Bioenerg. 1019, 59–66.
572 Judd, S., van den Broeke, L.J.P., Shurair, M., Kuti, Y., Znad, H., 2015. Algal
573 remediation of CO2 and nutrient discharges: A review. Water Res. 87, 356–366.
574 Juri, S., Vojta, L., Fulgosi, H., 2013. Electron Transfer Routes in Oxygenic
575 Photosynthesis: Regulatory Mechanisms and New Perspectives. Photosynthesis.
576 Koku, H., Erolu, I., Gündüz, U., Yücel, M., Türker, L., 2002. Aspects of the metabolism
577 of hydrogen production by Rhodobacter sphaeroides. Int. J. Hydrogen Energy 27,
578 1315–1329.

579 Lamperti, C., Naini, A., Hirano, M., De Vivo, D.C., Bertini, E., Servidei, S., Valeriani,
580 M., Lynch, D., Banwell, B., Berg, M., Dubrovsky, T., Chiriboga, C., Angelini, C.,
581 Pegoraro, E., DiMauro, S., 2003. Cerebellar ataxia and coenzyme Q10 deficiency.
582 Neurology 60, 1206–1208.
583 Lee, W.S., Chua, A.S.M., Yeoh, H.K., Ngoh, G.C., 2014. A review of the production
584 and applications of waste-derived volatile fatty acids. Chem. Eng. J. 235, 83–99.
585 Lemos, P.C., Serafim, L.S., Reis, M.A.M., 2006. Synthesis of polyhydroxyalkanoates
586 from different short-chain fatty acids by mixed cultures submitted to aerobic
587 dynamic feeding. J. Biotechnol. 122, 226–238.
588 Lu, H., Han, T., Zhang, G., Ma, S., Zhang, Y., Li, B., Cao, W., 2018. Natural light-
589 micro aerobic condition for PSB wastewater treatment: a flexible, simple, and
590 effective resource recovery wastewater treatment process. Environ. Technol.
591 (United Kingdom) 39, 74–82.
592 Lu, H., Zhang, G., He, S., Peng, C., Ren, Z., 2020. Production of photosynthetic
593 bacteria using organic wastewater in photobioreactors in lieu of a culture medium
594 in fermenters: From lab to pilot scale. J. Clean. Prod. 120871.
595 Lu, H., Zhang, G., Wan, T., Lu, Y., 2011. Influences of light and oxygen conditions on
596 photosynthetic bacteria macromolecule degradation: Different metabolic
597 pathways. Bioresour. Technol.
598 Madukasi, E.I., Dai, X., He, C., Zhou, J., 2010. Potentials of phototrophic bacteria in
599 treating pharmaceutical wastewater. Int. J. Environ. Sci. Technol. 7, 165–174.
600 McCully, A.L., Onyeziri, M.C., Lasarre, B., Gliessman, J.R., McKinlay, J.B., 2020.

601 Reductive tricarboxylic acid cycle enzymes and reductive amino acid synthesis
602 pathways contribute to electron balance in a rhodospirillum rubrum calvin-cycle
603 mutant. Microbiol. (United Kingdom) 166, 199–211.
604 Meng, F., Yang, A., Zhang, G., Wang, H., 2017. Effects of dissolved oxygen
605 concentration on photosynthetic bacteria wastewater treatment: Pollutants
606 removal, cell growth and pigments production. Bioresour. Technol. 241, 993–997.
607 Nakajima, F., Kamiko, N., Yamamoto, K., 1997. Organic wastewater treatment without
608 greenhouse gas emission by photosynthetic bacteria. Water Sci. Technol. 35, 285–
609 291.
610 Park, Y.C., Kim, S.J., Choi, J.H., Lee, W.H., Park, K.M., Kawamukai, M., Ryu, Y.W.,
611 Seo, J.H., 2005. Batch and fed-batch production of coenzyme Q10 in recombinant
612 Escherichia coli containing the decaprenyl diphosphate synthase gene from
613 Gluconobacter suboxydans. Appl. Microbiol. Biotechnol. 67, 192–196.
614 Peng, L., Shimizu, K., 2003. Global metabolic regulation analysis for Escherichia coli
615 K12 based on protein expression by 2-dimensional electrophoresis and enzyme
616 activity measurement. Appl. Microbiol. Biotechnol. 61, 163–178.
617 Ponsano, E.H.G., Paulino, C.Z., Pinto, M.F., 2008. Phototrophic growth of Rubrivivax
618 gelatinosus in poultry slaughterhouse wastewater. Bioresour. Technol. 99, 3836–
619 3842.
620 Puyol, D., Barry, E.M., Hülsen, T., Batstone, D.J., 2017. A mechanistic model for
621 anaerobic phototrophs in domestic wastewater applications: Photo-anaerobic
622 model (PAnM). Water Res. 116, 241–253.

623 Rombouts, J.L., Mos, G., Weissbrodt, D.G., Kleerebezem, R., Van Loosdrecht, M.C.M.,
624 2018. Diversity and metabolism of xylose and glucose fermenting microbial
625 communities in sequencing batch or continuous culturing. FEMS Microbiol. Ecol.
626 95.
627 Saejung, C., Thammaratana, T., 2016. Biomass recovery during municipal wastewater
628 treatment using photosynthetic bacteria and prospect of production of single cell
629 protein for feedstuff. Environ. Technol. (United Kingdom) 37, 3055–3061.
630 Schagerl, M., Müller, B., 2006. Acclimation of chlorophyll a and carotenoid levels to
631 different irradiances in four freshwater cyanobacteria. J. Plant Physiol. 163, 709–
632 716.
633 Segura, P.C., De Meur, Q., Tanghe, A., Onderwater, R., Dewasme, L., Wattiez, R.,
634 Leroy, B., 2021. Effects of mixing volatile fatty acids as carbon sources on
635 rhodospirillum rubrum carbon metabolism and redox balance mechanisms.
636 Microorganisms 9.
637 Tang, K.H., Tang, Y.J., Blankenship, R.E., 2011. Carbon metabolic pathways in
638 phototrophic bacteria and their broader evolutionary implications. Front.
639 Microbiol. 2, 1–23.
640 Tian, Y., Yue, T., Yuan, Y., Soma, P.K., Lo, Y.M., 2010. Improvement of cultivation
641 medium for enhanced production of coenzyme Q10 by photosynthetic
642 Rhodospirillum rubrum. Biochem. Eng. J. 51, 160–166.
643 Wang, X., Carvalho, G., Reis, M.A.M., Oehmen, A., 2018. Metabolic modeling of the
644 substrate competition among multiple VFAs for PHA production by mixed

645 microbial cultures. J. Biotechnol. 280, 62–69.
646 Xu, J.Z., Zhang, J.L., Guo, Y.F., Jia, Q.D., Zhang, W.G., 2014. Heterologous
647 expression of escherichia coli fructose-1,6-bisphosphatase in corynebacterium
648 glutamicum and evaluating the effect on cell growth and l-lysine production. Prep.
649 Biochem. Biotechnol. 44, 493–509.
650 Yadav, G., Mishra, A., Ghosh, P., Sindhu, R., Vinayak, V., Pugazhendhi, A., 2021.
651 Technical, economic and environmental feasibility of resource recovery
652 technologies from wastewater. Sci. Total Environ. 796, 149022.
653 Yang, A., Peng, M., Zhang, G., Meng, F., Zhang, Y., Zou, Z., 2018. Effects of light-
654 oxygen conditions on microbial community of photosynthetic bacteria during
655 treating high-ammonia wastewater. Process Biochem.
656 Yu, S., Peng, L., Xu, Y., Song, S., Xie, G.J., Liu, Y., Ni, B.J., 2021. Optimizing light
657 sources for selective growth of purple bacteria and efficient formation of value-
658 added products. J. Clean. Prod. 280, 124493.
659 Zhi, R., Cao, K., Zhang, G., Zhu, J., Xian, G., 2020. Zero excess sludge wastewater
660 treatment with value-added substances recovery using photosynthetic bacteria. J.
661 Clean. Prod. 250, 119581.
662 Zhi, R., Yang, A., Zhang, G., Zhu, Y., Meng, F., Li, X., 2019. Effects of light-dark
663 cycles on photosynthetic bacteria wastewater treatment and valuable substances
664 production. Bioresour. Technol. 274, 496–501.
665 Zhou, Q., Zhang, P., Zhang, G., 2014. Biomass and carotenoid production in
666 photosynthetic bacteria wastewater treatment: Effects of light intensity. Bioresour.

667 Technol. 171, 330–335.

668