1   **Comparative evaluation of continuous piggery wastewater treatment**

2   **in open and closed purple phototrophic bacteria-based**

3   **photobioreactors.**

4

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Abstract

Purple phototrophic bacteria (PPB) represent an innovative approach for wastewater treatment with a high metabolic plasticity, able to grow under aerobic and anaerobic conditions. This study comparatively assessed the long-term performance (450 days of operation) of an open and closed PPB-based photobioreactor treating of piggery wastewater (PWW). The influence of wastewater dilution, illuminated area to volume ratio, biomass settling and recirculation, and infrared light intensity on wastewater treatment was evaluated at 7 days of hydraulic retention time. An increase in PWW dilution from 4 to 8 folds did not entail higher TOC removal efficiencies (REs) in the open photobioreactor (87% versus 89%), but a significant increase in the closed photobioreactor (from 73% to 80%). The increase in the illuminated area to volume ratio increased TN-REs up to 99% and 49% in the open and closed photobioreactor, respectively, with a concomitant increase in the temperature of both systems. However, temperature control did not mediate a significant enhancement in PWW treatment. Biomass settling and recirculation resulted in higher TN-REs (80%) and TOC-REs (90%) in the closed photobioreactor. The increase in infrared radiation from 100 to 300 W m^{-2} fostered PPB growth. High water evaporation losses (deteriorating effluent quality) were recorded in the open photobioreactor, where carbon dioxide and ammonia stripping were identified as the main pathways supporting carbon and nitrogen removal.

Keywords: Nutrient removal; PPB; Purple non-sulphur bacteria; Photosynthetic bacteria; Swine manure.

1. Introduction

High strength wastewaters such as those produced by intensive animal husbandry represent a severe environmental problem that is also limiting the growth of this economic sector in Europe. Piggery wastewater (PWW) is characterized by a high content of particulate and dissolved organic carbon, nitrogen (mainly in the form of NH_4^+) and phosphorous due to the limited use of water during farming [1–3], which can severely damage water bodies and soil if not properly managed [4]. In this context, photosynthetic microorganisms have been proposed as cost-effective platforms for the removal of nutrients and carbon from PWW [3,5–9]. Photosynthetic microorganisms represent unique microbial cell factories due to their ability to fix carbon and nutrients using energy from solar light, via oxygenic and anoxygenic photosynthesis in the case of microalgae and purple phototrophic bacteria (PPB), respectively [10,11].

PPB exhibit a superior metabolism compared to other photosynthetic microorganisms (microalgae and green sulfur bacteria), which is characterized by high growth rates [12], tolerance to low temperatures [13], and ability to assimilate multiple substrates and grow in any kind of wastewater [14]. PPB are among the most metabolically versatile microorganisms that can grow under chemotrophic, phototrophic and mixotrophic condition. Under aerobic chemotrophic metabolism, pollutant bio-degradation occurs mainly by oxidative phosphorylation [15]. However, the competition with aerobic chemoheterotrophic bacteria in aerated tank treating wastewater decreases the concentration of PPB [16], whose metabolism is favored at low oxygen concentrations. On the other hand, PPB are able to grow phototrophically using near infrared light energy as energy source under anaerobic conditions [17]. PPB can fix CO_2 via the Calvin-Benson-Bassham pathway, and encode the Embden-Meyerhof pathway, tricarboxylic

74 acid cycle (TCA), pentose phosphate and multiple aromatic biodegradation pathways

75 [17]. In addition, PPBs can assimilate all forms of nitrogen (including N_2), which supports

76 their high potential for the assimilation of nutrients from wastewater [14]. Finally, PPB

77 biomass is rich in value-added products such single cell protein, pigments (carotenoids

78 and bacteriochlorophylls), biopolymers (PHA), antimicrobial agents, pantothenic acid,

79 coenzyme Q10 and amino acid 5-ALA [11,18,19].

80

81 The photo-anaerobic membrane bioreactor has been the main configuration used for the

82 treatment of domestic, dairy, food and poultry processing wastewater with PPB [10,20–

83 22]. However, despite their efficiency [23], the use of membranes hinders the industrial

84 scale up of this bioreactor configuration due to the need for complex control systems and

85 the increase in operating costs [24]. In this context, the engineering of simple and cost-

86 effective photobioreactor configurations remains an unresolved challenge for PPB-based

87 treatment PWW. Shallow covered ponds with biomass settling and recycling represent a

88 cost-effective but poorly explored photobioreactor configuration to treat wastewater

89 using PPB. The key operational conditions determining the performance of PPB-ponds

90 with biomass settling and recycling need to be also investigated.

91

92 In this work, the long-term performance of two PPB-based photobioreactor

93 configurations (open and closed) during the treatment of PWW was evaluated. The

94 influence of wastewater dilution, illuminated area to volume ratio, biomass settling and

95 recirculation, and infrared light intensity on the removal of carbon and nitrogen was

96 comparatively assessed.

97

98 **2. Materials and Methods**

99 *2.1. Inoculum and piggery wastewater*

100 The inoculum of PPB was taken from a batch cultivation of a previous study with

101 *Rhodopseudomonas* as the dominant genus [6]. Inoculum of PPB was carried out in 1.2

102 L gas-tight bottles (Afora, Spain) with 600 mL of four times diluted piggery wastewater

103 under a N_2 atmosphere. The PPB culture was incubated at room temperature (25 ± 1 °C)

104 under magnetic mixing (300 rpm) and an infrared lighting (50 W m^{-2}). Centrifuged PWW

105 was obtained from a pig farm in Segovia (Spain) and maintained at 4 °C prior use. The

106 PWW was further centrifuged (10,000 rpm, 10 min) prior to use. The characteristics of

107 the PWW used in the experiments was: total organic carbon concentration (TOC) of 9.4

108 $\pm$ 1.2 g L^{-1}, total carbon concentration (TC) of 10.5 ± 1.0 g L^{-1}, inorganic carbon

109 concentration (IC) of 1.0 ± 0.3 g L^{-1}, total nitrogen concentration (TN) of 3.0 ± 0.4 g L^{-1}

110 and total suspended solids concentration (TSS) of 10.5 ± 2.2 g L^{-1}.

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112 2.2. *Continuous PWW biodegradation in open and closed photobioreactors*

113 The experimental set-up consisted of two rectangular photobioreactors (20 cm length $\times$

114 10 cm width $\times$ 15 cm depth; 3 L of working volume) (Fig. 1) constructed with transparent

115 covers and interconnected to 1 L conical settlers. The covers were placed either 2 cm

116 above the top of the open photobioreactor (PBR) to favor air supply or at the cultivation

117 broth surface level in the closed PBR to guarantee anaerobic conditions. The systems

118 were agitated with two submerged centrifugal pumps. The PBRs were illuminated at 100

119 W m^{-2} (stages I-V) or 300 W m^{-2} (stages VI) for 12 h a day using an infrared LED panel

120 (diodes OSLUX® SFH 4780S and SFH 4715AS, OSRAM, Germany) located 20 cm

121 above the surface of the cultivation broth. The PBRs were initially operated at a hydraulic

122 retention time (HRT) of 7 days using 4 folds diluted PWW (stage I). An aliquot of 56 and

123 12 mL in stage I-II and III-IV respectively, was daily drawn from the bottom of the settler

124 to waste the settled biomass. The dilution of the PWW was increased to 8 fold by day 102

125 and maintained for the rest of the experiment (stage II). In stage III, the illuminated area

126 to volume ratio was increased from 66.7 $cm^2 L^{-1}$ to 133 $cm^2 L^{-1}$ by reducing the working

127 volume of the PBRs to 1.5 L (via a reduction in the PBR depth from 15 cm to 7.5 cm). A

128 cooling system based on PBR jacketing was implemented in both PBRs by day 239

129 (beginning of stage IV) to maintain similar temperatures as in stage II due to the increase

130 in temperature mediated by the heat generated by the submerged centrifugal pumps in the

131 new working volume. In stage V, biomass recirculation from the bottom of the conical

132 settlers was implemented at a rate of 167 mL d^{-1}. Finally, the intensity of the infrared

133 radiation was increased to 300 W m^{-2} during stage VI (Table 1).

134 <Figure 1>

135 <Table 1>

136 Samples of 20 mL from the centrifuged PWW (raw influent), cultivation broth and

137 effluent of the open and closed PBRs were collected systematically twice a week to

138 analyze pH and TOC, TC, IC, TN, NH_4^+ and TSS concentrations. The dissolved oxygen

139 concentration (DO) and temperature in the culture broths of the PBRs was in-situ

140 measured twice a week. The culture absorbance in the each PBR was also measured twice

141 a week. In addition, an aliquot of biomass from each steady state (10 min at 10,000 rpm)

142 was centrifuged, washed and dried to analyze its elemental composition (C, H, O and N

143 content).

144 The removal efficiencies (REs), expressed in percentage, of TOC, TN and TSS were

145 calculated according to the following equation:

146
$$RE\ (\%) = \frac{(C_{inf} \cdot Q_{inf}) - (C_{eff} \cdot Q_{eff})}{(C_{inf} \cdot Q_{inf})} \cdot 100$$

147 Where C_{inf} and C_{eff} correspond to the concentration of TOC, TN and TSS in the piggery

148 wastewater influent and effluent of the PBRs, respectively, while Q_{inf} and Q_{eff} correspond

6

149 to the flowrate in the piggery wastewater influent and effluent of the PBRs, respectively.

150 The removal efficiencies were calculated in steady state for each PBR.

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152 *2.3. Analytical methods*

153 The pH determinations were conducted with a pH 510 pHmeter (Cyberscan,

154 Netherlands). A ProfiLine 3320 meter coupled with a sensor CellOx 325 (WTW,

155 Germany) was used to measure the DO and temperature. Infrared light intensity was

156 determined with a PASPort PS-2148 IR sensor (PASCO, USA). Measurements of

157 dissolved TOC, TC, IC and TN concentrations were carried out in a TOC-VCSH

158 instrument (Shimadzu, Japan) coupled with a TNM-1 unit. The spectrum of absorbance

159 (350–850 nm) of the cultivation broth was analyzed in a spectrophotometer UV-2550

160 (Shimadzu, Japan). NH_4^+ analysis was conducted using a sensor Orion Dual Star

161 (ThermoScientific, The Netherlands). The elemental composition was analyzed using an

162 elemental analyzer EA Flash 2000 equipped with a TCD detector (Thermo Fisher

163 Scientific). Finally, the quantification of TSS concentration was performed following the

164 procedure of Standard Methods [25].

165

166 *2.4. Statistical analysis*

167 Statgraphics Centurion version 18 was used for the analysis of variance (ANOVA) and a

168 Tukey test carried out to identify the significance of the values obtained, comparisons

169 with a value of $p < 0.05$ were considered significant. Performed to the experimental data

170 obtained under steady state.

171

172 **3. Results and Discussion**

173 3.1. *Environmental parameters*

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174   Temperatures in the closed PBR were higher than those in the open PBR as a result of the

175   evaporation-based heat losses in the latter, with average values of 32 ± 1 °C and 36 ± 2

176   °C in the open and closed PBR, respectively. Temperatures of 32 ± 1 °C and 35 ± 3 °C in

177   stage I, and 31 ± 1 °C and 33 ± 1 °C in stage II, were recorded in the open and closed

178   PBR, respectively (Table 2). The heat generated by the submerged mixing pumps resulted

179   in a high increase in temperatures in the closed PBR in stage III, where temperatures

180   reached 40 °C occasionally due to the low PBR volumes compared to stage I and II (Fig.

181   S1A). Likewise, an increase in temperature was recorded in stage VI in both PBRs ($34 \pm$

182   0 °C in open the PBR and 40 ± 2 °C in the closed PBR) due to the increase in IR radiation.

183   The optimal growth temperature in biological wastewater treatment is species specific.

184   For instance, *Rhodopseudomonas palustris* exhibits optimum growth at 37 °C, while *R.*

185   *capsulatus* and *R. spheroids* growth rate peaks at 30 °C [26]. Although the temperatures

186   recorded in this work were high, the communities present in both PBRs were able to adapt

187   and support an efficient removal of carbon and nutrients as described below. These high

188   temperatures prevailing in the cultivation broth mediated the high evaporation rates

189   observed in the open PBR, which accounted for 58, 97, 84, 89 and 99% of the inlet flow

190   in stages I-II, III, IV, V and VI, respectively. This resulted in low effluent flowrates in

191   the stages with higher temperature (stage III and VI). The loss of water in open ponds

192   devoted to photosynthetic microorganisms cultivation due to evaporation entails higher

193   operational costs (due to the need for water make-up), and can also increase the risk of

194   contamination with unwanted microorganisms [27]. In addition, the high evaporation rate

195   herein recorded in the open PBR resulted in the concentration of the effluent pollutants,

196   thus impacting on the removal efficiencies of the open PBR [6]. On the other hand,

197   although the temperatures recorded in the closed PBR were higher than in the open PBR,

198   the evaporation rates averaged of 10, 30, 14, 17 and 11% in stages I-II, III, IV, V and VI,

199   respectively, and likely occurred in the open settler interconnected to the PBR. This PBR

200   configuration favors the recovery of treated water, which is central in areas with severe

201   water stress such as the Mediterranean region.

202   <Table 2>

203   The dissolved oxygen (DO) concentration remained very low in both PBRs regardless of

204   the operational conditions (Fig. S1B), with average values of 0.03 ± 0.01 and 0.02 ± 0.01

205   mg O_2 L^{-1} in the open and closed PBR, respectively. The oxygen diffusing from the open

206   atmosphere into the open PBR cultivation broth was rapidly consumed by

207   chemoheterotrophic PPB or other aerobic heterotrophic bacteria for the degradation of

208   organic matter. The low oxygen concentration in the closed PBR, caused by the negligible

209   oxygen input into this system and the high organic matter content of the PWW,

210   maintained strict anaerobic conditions in the cultivation broth. In this context, low

211   dissolved oxygen concentration (DO < 0.5 mg L^{-1} which is considerable higher than the

212   values detected in both reactors) promote the activity of the enzyme dehydrogenase [28],

213   which favors the degradation of organic compounds through the TCA cycle in PPB.

214   According to previous studies, aerobic conditions during wastewater treatment limit the

215   development of PPB [16].

216   The pH of the PWW was 7.8 ± 0.3, while the pH of the cultivation broths remained very

217   stable along the different stages tested (Fig. S1C), with pH values of 8.7 ± 0.1 and $8.3 \pm$

218   0.2 in the open and closed PBRs, respectively. The higher pH in the open PBR was likely

219   due to the stripping of CO_2 from the cultivation broth to the open atmosphere. Likewise,

220   the degradation of volatile fatty acids (VFAs) by PPB likely induced the increase in pH

221   observed in both PBRs due to the consumption of organic acids by VFAs catabolic

222   pathways, which also enhanced the fixation of the dissolved CO_2 [12]. The optimal pH

223   range described for PPB is 6.0 to 9.0 [14], which matched the pH recorded in both PBRs.

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3.2. *Wastewater treatment performance*

3.2.1. *Carbon removal*

The TOC concentration in the influent (PWW) was maintained constant in stage I at 2.7 $\pm$ 0.2 g L^{-1} TOC and 1.2 $\pm$ 0.1 g L^{-1} in the following stages (II-VI) (Fig. 2A). High carbon removal efficiencies of 87 $\pm$ 1% and 73 $\pm$ 2% were recorded under steady state in stage I in the open and closed PBR, respectively (Fig. 3A). However, a limited PPB growth was observed in both PBRs as shown by gradual disappearance of the characteristic purple-red color in the cultivation broths. Independent measurements of infrared light penetration in 4 fold diluted PWW showed that the photic zone was only ~ 1 cm due to the high wastewater turbidity. The limited growth of PPB was attributed to the low penetration of IR radiation in both PBRs, which resulted in a reduced capacity of PPBs to obtain energy from anoxygenic photosynthesis and to degrade organic carbon. In this context, PPB in the absence of or under limited infrared light supply are not able to compete with other chemotrophic bacteria as a result of their less efficient fermentative metabolism [16]. An increase in PWW dilution resulted in a significant increase in TOC-REs in stage II up to 89 $\pm$ 1% and 80 $\pm$ 2% in the open and closed PBR, respectively. The increase in PWW dilution enhanced the penetration of IR radiation, doubling the photic zone depth and thus favoring the growth of PPB and the removal of carbon. In this context, the increase in the illuminated area to volume ratio caused by the reduction in the depth of both PBRs from 15 to 7.5 cm in stage III significantly favored the removal of carbon, with TOC-REs of 99 $\pm$ 0% and 84 $\pm$ 2% in the open and closed PBR, respectively. This increase in the illuminated area to volume ratio also mediated an increase in the temperature of the cultivation broths and in the water evaporation rates, which suggests that the improvement in TOC removal in the open PBR was not only due to an enhanced PPB

249   activity but also to a higher stripping of carbon dioxide. An environmental benefit derived

250   from the implementation of closed PBRs is the reduction in gas emissions into the

251   atmosphere, which prevents the release of CO_2 and CH_4 potentially generated under

252   anaerobic conditions. Temperature control in stage IV did not favor TOC-REs in the open

253   PBR, which decreased to $91 \pm 1\%$, but increased TOC-REs by 4% in the closed PBR

254   compared to the previous stage (III), likely due to the increase in PPB activity. The

255   recirculation of the settled biomass in stage V resulted in an improvement in TOC-REs

256   up to $96 \pm 1\%$ and $90 \pm 1\%$ in the open and closed PBRs, respectively. This improvement

257   was likely due to the increase in PPB biomass in the PBRs, which boosted the removal of

258   the carbon present in PWW. Finally, the increase in IR radiation intensity in stage VI

259   from 100 to 300 W m^{-2} (Fig. S1D) did not significantly improve the removal of carbon in

260   the closed PBR (TOC-REs of $91 \pm 1\%$), but resulted in a complete TOC removal in the

261   open PBR due to the increase in the evaporation rate. The results herein obtained

262   confirmed the consistent removals of organic matter by PPB and were in agreement with

263   the TOC-REs of 87, 84 and 77% recorded by García el al. (2019) in an open

264   photobioreactor treating PWW at HRTs of 10.6, 7.6 and 4.1 days respectively, using 20

265   fold diluted PWW [6].

266   <Figure 2>

267   <Figure 3>

268   A preliminary carbon mass balance revealed that the closed PBR supported higher carbon

269   recoveries than the open PBR (e.g. 82% vs 52% in stage I). Overall, carbon recovery in

270   the closed PBR was 36% higher than in the open PBR. The main mechanism of carbon

271   removal in the open PBR was stripping, and assimilation in the closed PBR, which agreed

272   with the water evaporation rates recorded in both systems.

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274   3.2.2. *Nitrogen removal*

275   In stage I, TN-REs of 78 ± 1% and 21 ± 3% were recorded in the open and closed PBR,

276   respectively (Fig. 3B). Similarly, the removal of ammonia was higher in the open PBR

277   (0.58 g L^{-1} removed) compared to that in the closed PBR (0.21 g L^{-1} removed). A slight

278   decrease in TN-REs to 72 ± 2% and 17 ± 4% in the open and closed PBR, respectively,

279   was observed in stage II along with the increase in PWW dilution. The high TN removal

280   observed in the open PBR in stages I and II can be explained by the active ammonium

281   stripping from the PBR to the atmosphere [8] and by the consumption of nitrogen by other

282   microorganisms different from PPB. On the other hand, the low TN removal recorded in

283   the closed PBR was attributed mainly to the assimilation of NH_4^+ into biomass (in the

284   form of microbial protein), since the air-tight PBR cover prevented ammonium stripping

285   in this type of configuration. In stage III an increase of TN-REs was recorded with 99 ±

286   0% and 49 ± 6% in the open and closed PBR, respectively, which was likely induced by

287   the higher PPB growth favored by the increased illuminated area to volume ratio in both

288   PBRs. The decrease in temperatures during stage IV resulted in TN-REs of 95 ± 1% and

289   43 ± 7% in the open and closed PBRs, respectively. The effluent obtained during stage

290   IV presented low concentrations of NH_4^+ (0.00 g L^{-1} in the open PBR and 0.17 g L^{-1} in

291   the closed PBR). Interestingly, the implementation of the recirculation of the settled

292   biomass in stage V brought about higher TN-REs of 98 ± 0% in the open PBR and 80 ±

293   4% in the closed PBR. The analysis of the concentrations of ammonium during stage V

294   was not possible due to a failure of the NH_4^+ electrode (Table 2). Finally, the increase in

295   IR radiation during stage VI supported TN-REs and ammonium effluent concentrations

296   of 100 ± 0% and 0.02 g NH_4^+ L^{-1} in the open PBR, and 79 ± 2% and 0.08 g NH_4^+ L^{-1} in

297   the closed PBR. Removals efficiencies of 65% for total nitrogen and 68% for ammonium

298   have been reported in an open photobioreactor treating PWW with PPB at a HRT of 7.6

299 days [6]. Ammonia is the main form of nitrogen present in PWW, which can be

300 assimilated by PPB through glutamate metabolism and subsequently used in protein

301 synthesis. This metabolic capacity is present in PPBs species such as *R. palustris*, *R*

302 *capsulatus* and *R. sphaeroides*. During stages V and VI, 0.29 ± 0.02 and 0.27 ± 0.02 g N

303 L^{-1} were removed in the open and closed PBR, respectively (Fig. 2B), which lies within

304 the 3-8000 mg L^{-1} range described in literature studies assessing nitrogen removal by PPB

305 [28]. Nitrogen removal could be improved by increasing the C:N ratio [12] in PWW using

306 C-rich wastewaters to support a complete assimilation of the nitrogen present in PWW.

307

308 3.3. *Concentration and elemental composition of biomass*

309 Biomass concentration in the open PBR increased during the first 50 days from 1.7 g TSS

310 L^{-1} up to 5.6 g TSS L^{-1} in stage I, along with a rapid disappearance of the purple-red color

311 in the cultivation broth, suggesting an adaptation of other microbial communities.

312 Interestingly, biomass concentration in the open PBR gradually decreased to 3.8 g TSS

313 L^{-1} by the end of stage I likely due to natural cell death or the toxic effects of the

314 accumulated PWW contaminants (Fig. 4A). High PWW loads have been reported as

315 harmful to the growth of PPB [7,29], PWW dilution being identified as an operational

316 strategy to decrease the turbidity in the cultivation broth, favoring the penetration of the

317 radiation and decreasing the toxic effects of NH_4^+. A stable biomass concentration of 2.0

318 ± 0.3 g TSS L^{-1} was recorded in the open PBR during stage II, while the increase in

319 temperature and in the evaporation rate entailed an increase in biomass concentration up

320 to 4.9 ± 0.6 g TSS L^{-1} in stage III. Finally, a gradual decrease in biomass concentration in

321 the open PBR to 4.2 ± 0.2 g TSS L^{-1}, 2.4 ± 0.7 g TSS L^{-1} and 2.0 ± 0.4 g TSS L^{-1} was

322 recorded in stages VI, V and VI (Fig. 4A). High TSS-REs were recorded in stage I ($75 \pm$

323 2%) and in the later stages in the open PBR (up 80%) (Fig. 3C). Biomass concentration

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324 was stable in the closed PBR, with 2.4 ± 0.3 g TSS L^{-1} in stage I, and 1.3 ± 0.2 g TSS L^{-1}

325 ^{1} in stage II-VI, with transient increase in stage V caused by the recirculation of biomass

326 (Fig. 4B). Interestingly, the ratio of culture absorbance at 808 nm and TSS concentration

327 was similar in both PBRs during stages I-V, but significantly higher in the closed PBR

328 when infrared radiation was increased from 100 to 300 W m^{-2} (0.013 ± 0.002 in the open

329 PBR to 0.021 ± 0.001 in the closed PBR). TSS-REs in the closed PBR varied from 51 up

330 to 64% (Fig. 3C). The high biomass concentration generated in the open PBR, after stage

331 III did not settle completely, thus increasing the TSS concentrations recorded in the

332 effluent to values similar to the TSS concentrations present in the PWW and also. This

333 fact was also fostered by the low volume of effluent mediated by the high water

334 evaporations prevailing in the latter stage. Interestingly, the lower biomass concentration

335 present in the closed PBR compared to the open PBR was able to removed a similar

336 concentrations of pollutants and generate an effluent with lower TSS. These differences

337 in biomass concentration between both PBRs could be explained by higher water

338 evaporation rate recorded in the open PBR, which indirectly increase the TSS in the PBR.

339 Finally, it should be highlighted that PPB biomass contain high value-added products

340 such single cell protein, pigments, pantothenic acid and coenzyme Q10 [14]. In addition,

341 PPB biomass can be used as animal or fish feed [30] and as a bio-fertilizer, promoting

342 plant growth and boosting the resistance to environmental stresses by accumulation of

343 polyphosphate and synthesizing plant growth-promoting factors [31].

344 <Figure 4>

345 The C, N, H and S content in the PPB biomass averaged $44.3 \pm 1.7\%$, $7.1 \pm 0.8\%$, $6.4 \pm$

346 0.3% and $0.4 \pm 0.3\%$ in the open PBR and $49.8 \pm 0.9\%$, $8.2 \pm 0.4\%$, $7.6 \pm 0.2\%$ and 0.7

347 $\pm 0.2\%$ in the closed PBR, respectively. The PPB biomass composition was similar to the

348 values reported by [32], who recorded a C, N and H content of 52.1%, 10.7% and 8.4%,

349 respectively, in the biomass generated a tubular PBR inoculated with *Rhodopseudomonas*

350 *palustris* strain 42OL.

351 The nitrogen mass balance conducted in the PBRs revealed an overall nitrogen recovery

352 of 67% in the closed PBR, versus 20% in the open PBR. NH_3 stripping was identified as

353 the main nitrogen removal mechanisms in the open PBR, which agreed with the high

354 water evaporation rates recorded in this system.

355

356 The lower investment cost and high nutrient removal capacity constitute the main

357 advantages of open-PBRs [33]. However, the high rates of evaporation and CO_2/NH_3

358 stripping could eventually jeopardize their environmental performance [29,33].

359 Furthermore, culture contamination by other microorganisms is difficult to control in

360 open-PBRs. These limitations are partially mitigated in closed-PBR, where PPB growth

361 and nutrient recovery are also maximized (Table 3). The scalability of the technology is

362 technically feasible in both types of photobioreactors [33]. However, more research is

363 still required to assess microbial competition with other photosynthetic microorganisms

364 in outdoor systems.

365   <Table 3>

366 The evaluation of the scalability of this technology remains the main challenge for the

367 future, with successful case studies such as the work of Lu et al., (2019) where a 240 L

368 reactor was operated with promising resource recovery efficiencies [34]. In addition, the

369 technical and economic viability of the extraction of high added value compounds from

370 PPB in the context of the creation of a circular economy in the water sector remains

371 unexplored.

372

373 **4. Conclusions**

This study confirmed the long-term efficiency of PPB-based piggery wastewater treatment. The open PBR always supported higher TOC, TN and TSS removals than the closed PBR, which was mediated by the larger contribution of abiotic mechanisms such as CO_2 and NH_3 stripping. The decrease in PWW load did not entail an enhancement in process performance in both PBRs, while the increase in the illuminated area to volume ratio induced higher TOC and TN removals. Biomass settling and recirculation resulted in enhanced nitrogen removals. Finally, the increase in infrared radiation from 100 to 300 W m^{-2} favored PPB growth. The high water evaporation losses in the open PBR resulted in a significant deterioration of the effluent quality as a result of pollutant pre-concentration. PWW dilution and operation with high illuminated area were key parameters that favored PPB growth in the closed PBR. In addition, this type of PBR configuration prevents high water evaporations and favors the dominance of PPB.

Supplementary Materials: Figure S1: Time course of the temperatures (A), dissolved oxygen concentration (B), pH (C) and IR radiation (D) during PWW biodegradation by PPB.

399 **References**

400 [1] Y. Yang, E. Lin, S. Sun, X. Tao, L. Zhong, K. Hu, Piggery wastewater treatment

401 by Acinetobacter sp. TX5 immobilized with spent mushroom substrate in a fixed-

402 bed reactor, Sci. Total Environ. 644 (2018) 1460–1468.

403 https://doi.org/10.1016/J.SCITOTENV.2018.07.076.

404 [2] G. Chen, J. Huang, X. Tian, Q. Chu, Y. Zhao, H. Zhao, Effects of influent loads

405 on performance and microbial community dynamics of aerobic granular sludge

406 treating piggery wastewater, J. Chem. Technol. Biotechnol. 93 (2018) 1443–

407 1452. https://doi.org/10.1002/jctb.5512.

408 [3] I. de Godos, S. Blanco, P.A. García-Encina, E. Becares, R. Muñoz, Long-term

409 operation of high rate algal ponds for the bioremediation of piggery wastewaters

410 at high loading rates, Bioresour. Technol. 100 (2009) 4332–4339.

411 https://doi.org/10.1016/J.BIORTECH.2009.04.016.

412 [4] M.J. Luján-Facundo, M.I. Iborra-Clar, J.A. Mendoza-Roca, M. Also-Jesús,

413 Alternatives for the management of pig slurry: Phosphorous recovery and biogas

414 generation, J. Water Process Eng. 30 (2019) 100473.

415 https://doi.org/10.1016/j.jwpe.2017.08.011.

416 [5] I. de Godos, V.A. Vargas, S. Blanco, M.C.G. González, R. Soto, P.A. García-

417 Encina, E. Becares, R. Muñoz, A comparative evaluation of microalgae for the

418 degradation of piggery wastewater under photosynthetic oxygenation, Bioresour.

419 Technol. 101 (2010) 5150–5158.

420 https://doi.org/10.1016/J.BIORTECH.2010.02.010.

421 [6] D. García, I. de Godos, C. Domínguez, S. Turiel, S. Bolado, R. Muñoz, A

422 systematic comparison of the potential of microalgae-bacteria and purple

423 phototrophic bacteria consortia for the treatment of piggery wastewater,

424 Bioresour. Technol. 276 (2019) 18–27.

425 https://doi.org/10.1016/J.BIORTECH.2018.12.095.

426 [7] D. Marín, E. Posadas, D. García, D. Puyol, R. Lebrero, R. Muñoz, Assessing the

427 potential of purple phototrophic bacteria for the simultaneous treatment of

428 piggery wastewater and upgrading of biogas, Bioresour. Technol. 281 (2019) 10–

429 17. https://doi.org/10.1016/J.BIORTECH.2019.02.073.

430 [8] L. de S. Leite, M.T. Hoffmann, L.A. Daniel, Microalgae cultivation for

431 municipal and piggery wastewater treatment in Brazil, J. Water Process Eng. 31

432 (2019) 100821. https://doi.org/10.1016/j.jwpe.2019.100821.

433 [9] L. de S. Leite, M.T. Hoffmann, L.A. Daniel, Coagulation and dissolved air

434 flotation as a harvesting method for microalgae cultivated in wastewater, J. Water

435 Process Eng. 32 (2019) 100947. https://doi.org/10.1016/j.jwpe.2019.100947.

436 [10] T. Hülsen, K. Hsieh, S. Tait, E.M. Barry, D. Puyol, D.J. Batstone, White and

437 infrared light continuous photobioreactors for resource recovery from poultry

438 processing wastewater – A comparison, Water Res. 144 (2018) 665–676.

439 https://doi.org/10.1016/J.WATRES.2018.07.040.

440 [11] A. Talaiekhozani, S. Rezania, Application of photosynthetic bacteria for removal

441 of heavy metals, macro-pollutants and dye from wastewater: A review, J. Water

442 Process Eng. 19 (2017) 312–321. https://doi.org/10.1016/j.jwpe.2017.09.004.

443 [12] T. Hülsen, D.J. Batstone, J. Keller, Phototrophic bacteria for nutrient recovery

444 from domestic wastewater, Water Res. 50 (2014) 18–26.

445 https://doi.org/10.1016/J.WATRES.2013.10.051.

446 [13] T. Hülsen, E.M. Barry, Y. Lu, D. Puyol, D.J. Batstone, Low temperature

447 treatment of domestic wastewater by purple phototrophic bacteria: Performance,

448 activity, and community., Water Res. 100 (2016) 537–545.

449   https://doi.org/10.1016/J.WATRES.2016.05.054.

450 [14] H. Lu, G. Zhang, Z. Zheng, F. Meng, T. Du, S. He, Bio-conversion of

451   photosynthetic bacteria from non-toxic wastewater to realize wastewater

452   treatment and bioresource recovery: A review, Bioresour. Technol. 278 (2019)

453   383–399. https://doi.org/10.1016/J.BIORTECH.2019.01.070.

454 [15] H. Lu, G. Zhang, S. Dong, Quantitative study of PNSB energy metabolism in

455   degrading pollutants under weak light-micro oxygen condition, Bioresour.

456   Technol. 102 (2011) 4968–4973.

457   https://doi.org/10.1016/J.BIORTECH.2011.01.027.

458 [16] E. Siefert, R.L. Irgens, N. Pfennig, Phototrophic purple and green bacteria in a

459   sewage treatment plant., Appl. Environ. Microbiol. 35 (1978) 38–44.

460   http://www.ncbi.nlm.nih.gov/pubmed/623470 (accessed October 18, 2019).

461 [17] F.W. Larimer, P. Chain, L. Hauser, J. Lamerdin, S. Malfatti, L. Do, M.L. Land,

462   D.A. Pelletier, J.T. Beatty, A.S. Lang, F.R. Tabita, J.L. Gibson, T.E. Hanson, C.

463   Bobst, J.L.T. y Torres, C. Peres, F.H. Harrison, J. Gibson, C.S. Harwood,

464   Complete genome sequence of the metabolically versatile photosynthetic

465   bacterium *Rhodopseudomonas palustris*, Nat. Biotechnol. 22 (2004) 55–61.

466   https://doi.org/10.1038/nbt923.

467 [18] K. Cao, R. Zhi, G. Zhang, Photosynthetic bacteria wastewater treatment with the

468   production of value-added products: A review, Bioresour. Technol. (2019)

469   122648. https://doi.org/10.1016/J.BIORTECH.2019.122648.

470 [19] Q. Zhou, P. Zhang, G. Zhang, M. Peng, Biomass and pigments production in

471   photosynthetic bacteria wastewater treatment: Effects of photoperiod, Bioresour.

472   Technol. 190 (2015) 196–200.

473   https://doi.org/10.1016/J.BIORTECH.2015.04.092.

474 [20] T. Hülsen, E.M. Barry, Y. Lu, D. Puyol, J. Keller, D.J. Batstone, Domestic

475 wastewater treatment with purple phototrophic bacteria using a novel continuous

476 photo anaerobic membrane bioreactor, Water Res. 100 (2016) 486–495.

477 https://doi.org/10.1016/J.WATRES.2016.04.061.

478 [21] S. Chitapornpan, C. Chiemchaisri, W. Chiemchaisri, R. Honda, K. Yamamoto,

479 Organic carbon recovery and photosynthetic bacteria population in an anaerobic

480 membrane photo-bioreactor treating food processing wastewater, Bioresour.

481 Technol. 141 (2013) 65–74. https://doi.org/10.1016/j.biortech.2013.02.048.

482 [22] J. Kaewsuk, W. Thorasampan, M. Thanuttamavong, G.T. Seo, Kinetic

483 development and evaluation of membrane sequencing batch reactor (MSBR) with

484 mixed cultures photosynthetic bacteria for dairy wastewater treatment, J.

485 Environ. Manage. 91 (2010) 1161–1168.

486 https://doi.org/10.1016/j.jenvman.2010.01.012.

487 [23] L. Goswami, R. Vinoth Kumar, S.N. Borah, N. Arul Manikandan, K.

488 Pakshirajan, G. Pugazhenthi, Membrane bioreactor and integrated membrane

489 bioreactor systems for micropollutant removal from wastewater: A review, J.

490 Water Process Eng. 26 (2018) 314–328.

491 https://doi.org/10.1016/j.jwpe.2018.10.024.

492 [24] Z. Zhong, X. Wu, L. Gao, X. Lu, B. Zhang, Efficient and microbial communities

493 for pollutant removal in a distributed-inflow biological reactor (DBR) for treating

494 piggery wastewater, RSC Adv. 6 (2016) 95987–95998.

495 https://doi.org/10.1039/c6ra20777d.

496 [25] APHA, Standard Methods for the Examination of Water and Wastewater, 21st

497 ed. American Public Health Association, Washington, DC, USA., (2005).

498 [26] C.B. van Niel, The culture, general physiology, morphology, and classification of

499   the non-sulfur purple and brown bacteria, Bacteriol. Rev. 8 (1944) 1–118.

500   http://www.ncbi.nlm.nih.gov/pubmed/16350090 (accessed October 3, 2019).

501 [27] W.Y. Cheah, T.C. Ling, P.L. Show, J.C. Juan, J.S. Chang, D.J. Lee, Cultivation

502   in wastewaters for energy: A microalgae platform, Appl. Energy. 179 (2016)

503   609–625. https://doi.org/10.1016/j.apenergy.2016.07.015.

504 [28] F. Meng, A. Yang, G. Zhang, H. Wang, Effects of dissolved oxygen

505   concentration on photosynthetic bacteria wastewater treatment: Pollutants

506   removal, cell growth and pigments production, Bioresour. Technol. 241 (2017)

507   993–997. https://doi.org/10.1016/j.biortech.2017.05.183.

508 [29] C.A. Sepúlveda-Muñoz, I. de Godos, D. Puyol, R. Muñoz, A systematic

509   optimization of piggery wastewater treatment with purple phototrophic bacteria,

510   Chemosphere. 253 (2020) 126621.

511   https://doi.org/10.1016/j.chemosphere.2020.126621.

512 [30] M. Kobayashi, Y.T. Tchan, Treatment of industrial waste solutions and

513   production of useful by-products using a photosynthetic bacterial method, Water

514   Res. 7 (1973) 1219–1224. https://doi.org/10.1016/0043-1354(73)90075-4.

515 [31] M. Sakarika, J. Spanoghe, Y. Sui, E. Wambacq, O. Grunert, G. Haesaert, M.

516   Spiller, S.E. Vlaeminck, Purple non-sulphur bacteria and plant production:

517   benefits for fertilization, stress resistance and the environment, Microb.

518   Biotechnol. (2019) 1751-7915.13474. https://doi.org/10.1111/1751-7915.13474.

519 [32] P. Carlozzi, B. Pushparaj, A. Degl'Innocenti, A. Capperucci, Growth

520   characteristics of *Rhodopseudomonas palustris* cultured outdoors, in an

521   underwater tubular photobioreactor, and investigation on photosynthetic

522   efficiency, Appl. Microbiol. Biotechnol. 73 (2006) 789–795.

523   https://doi.org/10.1007/s00253-006-0550-z.

524   [33]   L. Christenson, R. Sims, Production and harvesting of microalgae for wastewater

525   treatment, biofuels, and bioproducts, Biotechnol. Adv. 29 (2011) 686–702.

526   https://doi.org/10.1016/j.biotechadv.2011.05.015.

527   [34]   H. Lu, M. Peng, G. Zhang, B. Li, Y. Li, Brewery wastewater treatment and

528   resource recovery through long term continuous-mode operation in pilot

529   photosynthetic bacteria-membrane bioreactor, Sci. Total Environ. 646 (2019)

530   196–205. https://doi.org/10.1016/j.scitotenv.2018.07.268.

531   [35]   A.P. Carvalho, L.A. Meireles, F.X. Malcata, Microalgal Reactors: A Review of

532   Enclosed System Designs and Performances, Biotechnol. Prog. 22 (2006) 1490–

533   1506. https://doi.org/10.1021/bp060065r.

534   [36]   O. Pulz, Photobioreactors: Production systems for phototrophic microorganisms,

535   Appl. Microbiol. Biotechnol. 57 (2001) 287–293.

536   https://doi.org/10.1007/s002530100702.

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547 **List of tables**

548

549 **Table 1.** Summary of photobioreactors operational period and conditions. Description of

550 the PWW dilution, culture depth, temperature control, biomass recirculation and infrared

551 radiation intensity.

552

553 **Table 2.** Summary of PWW composition, environmental variables, cultivation broth and

554 effluent characteristics in the open and closed photobioreactors during steady state along

555 the different operational stages (values represent average values ± standard deviation).

556

557 **Table 3**. Advantages and limitations of open and closed photobioreactors. Comparative

558 qualitative assessment of the economic and environmental performance of PPB-based

559 open and closed photobioreactors.

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List of figures

Figure 1. Schematic diagram of the PPB-based open and closed photobioreactors. The system is composed of a feeding tank, an open and closed PBR, settlers and effluent tanks.

Figure 2. Time course of the concentration of TOC (A) and TN (B) in the raw PWW and effluent from the open and closed PBR. This graph shows the long-term dynamics of the main pollutants degraded by PPB.

Figure 3. Removal efficiencies of TOC (A), TN (B) and TSS (C) in the open and closed photobioreactors during steady state in the different operational stages of piggery wastewater treatment by PPB. Removal efficiencies were estimated based on the concentrations and flow rates at the inlet and outlet of the PBRs.

Figure 4. Time course of TSS concentration in open (A) and closed (B) photobioreactors during the treatment of PWW by PPB. The concentration of solids in the influent, biomass in the culture broth and effluent are here depicted.

590   **Table 1.**

Stage	Operational days	Dilution of PWW	Depth of culture (cm) / area to volume ratio (cm^2 L^{-1})	Temperature control	Recirculation	Infrared radiation (W m^{-2})
I	101	4	15 / 66.7	No	No	100
II	67	8	15 / 66.7	No	No	100
III	70	8	7.5 / 133	No	No	100
IV	63	8	7.5 / 133	Yes	No	100
V	108	8	7.5 / 133	Yes	Yes	100
VI	53	8	7.5 / 133	Yes	Yes	300

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596 **Table 2.**

Parameters	PWW*	Stage I		Stage II		Stage III		Stage IV		Stage V		Stage VI	
		Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed
Temperature (°C)	-	31.7±1.4	35.3±2.5	30.7±1.2	32.7±1.4	33.2±1.2	36.4±2.5	30.5±1.0	34.5±1.7	32.0±1.3	36.3±1.8	34.4±0.06	39.9±1.6
pH	7.8±0.3	8.7±0.2	8.1±0.3	8.7±0.1	8.3±0.2	8.8±0.1	8.4±0.1	8.8±0.1	8.4±0.1	8.8±0.1	8.2±0.1	8.7±0.1	8.5±0.1
Radiation IR (W m^{-2})	-	97±7		97±4		93±4		99±7		101±4		293±7	
Evaporation (%)	-	58±1	10±2	58±1	10±2	97±3	30±18	84±15	14±6	89±6	17±7	99±3	11±5
TOC (g L^{-1})	1.18±0.15	0.89±0.06	0.84±0.06	0.35±0.05	0.29±0.04	0.34±0.03	0.27±0.02	0.66±0.07	0.17±0.03	0.42±0.04	0.15±0.03	0.50±0.05	0.14±0.03
IC (g L^{-1})	0.13±0.04	0.54±0.03	0.67±0.04	0.32±0.01	0.39±0.01	0.25±0.08	0.29±0.04	0.43±0.04	0.21±0.03	0.39±0.01	0.16±0.02	0.41±0.06	0.16±0.03
TN (g L^{-1})	0.38±0.05	0.50±0.03	0.81±0.03	0.27±0.01	0.36±0.01	0.12±0.03	0.27±0.03	0.12±0.02	0.22±0.05	0.08±0.01	0.10±0.03	0.09±0.02	0.10±0.0
NH$_4^+$ (g L^{-1})	0.40±0.12	0.43±0.05	0.80±0.05	0.24±0.02	0.37±0.03	0.04±0.05	0.17±0.07	0.00±0.00	0.17±0.02	-	-	0.02±0.01	0.08±0.02
TSS PBR (g L^{-1})	1.31±0.28	3.80±0.35	2.40±0.30	1.97±0.18	1.44±0.09	4.90±0.65	1.46±0.22	4.19±0.20	1.26±0.09	2.37±0.65	1.36±0.41	1.97±0.44	1.03±0.11
TSS Effluent (g L^{-1})		1.63±0.11	1.45±0.18	0.81±0.09	0.67±0.06	0.56±0.18	0.72±0.13	1.59±0.23	0.83±0.22	0.93±0.11	0.51±0.05	0.92±0.10	0.44±0.08

597 - Not applicable.

598 * 8 fold diluted PWW (in tap water).

599 TOC, IC, TN, NH$_4^+$ correspond to the concentration in the effluent of the photobioreactors.

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601   **Table 3.**

	Open-PBR	Closed-PBR	Reference
Nutrient removal	High	High	[29]
Nutrient recovery in biomass	Low	High	[29]
Biomass growth	Medium	High	[29]
Culture contamination	High	Low	[27,33,35,36]
Evaporation rate	High	Low	[27,33,35,36]
Culture control	Low	High	[33,35,36]
Environmental impact*	High	Low	[23,29,36]
Investment costs	Low	High	[33,35]
Scalability	High	Medium	[29,33]

602   * Atmospheric pollution by stripping CO_2 and NH_3.

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Figure 1.

IR radiation

Piggery wastewater

Pump

Open-PBR

Settler

Treated effluent

A

Closed-PBR

HRT = 7 days

Settler

Treated effluent

B

627   **Figure 2.**

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635 **Figure 3.**

638   **Figure 4.**

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