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Author(s)	Mohamed, A.Y.A.;Welles, L.;Siggins, A.;Healy, Mark G.;Brdjanovic, D.;Rada-Ariza, A.M.;Lopez-Vazquez, C.M.
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8 **Effects of Substrate Stress and Light Intensity on Enhanced** 9 **Biological Phosphorus Removal in a Photo-Activated Sludge System**

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11 A.Y.A. Mohamed ^{a, b, *}, L. Welles ^a, A. Siggins ^b, M.G. Healy ^b, D. Brdjanovic ^{a, c},

12 A.M. Rada-Ariza ^a, C.M. Lopez-Vazquez ^a

13 ^a Environmental Engineering and Water Technology Department. IHE Delft Institute for Water

14 Education, Westvest 7, 2611 AX Delft, The Netherlands.

15 ^b Civil Engineering and Ryan Institute, College of Science and Engineering, NUI Galway,

16 Republic of Ireland.

17 ^c Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ,

18 Delft, the Netherlands

19 * Corresponding author: a.yosif@hotmail.com

20

21 **Abstract**

22 Photo-activated sludge (PAS) systems are an emerging wastewater treatment technology where

23 microalgae provide oxygen to bacteria without the need for external aeration. There is limited

24 knowledge on the optimal conditions for enhanced biological phosphorus removal (EBPR) in

25 systems containing a mixture of polyphosphate accumulating organisms (PAOs) and microalgae.
26 This research aimed to study the effects of substrate composition and light intensity on the
27 performance of a laboratory-scale EBPR-PAS system. Initially, a model-based design was
28 developed to study the effect of organic carbon (COD), inorganic carbon (HCO_3) and ammonium-
29 nitrogen ($\text{NH}_4\text{-N}$) in nitrification deprived conditions on phosphorus (P) removal. Based on the
30 mathematical model, two different synthetic wastewater compositions (COD: HCO_3 : $\text{NH}_4\text{-N}$:
31 10:20:1 and 10:10:4) were examined at a light intensity of $350 \mu\text{mol m}^{-2} \text{sec}^{-1}$. Add to this, the
32 performance of the system was also investigated at light intensities: 87.5, 175, and $262.5 \mu\text{mol m}^{-2}$
33 sec^{-1} for short terms. Results showed that wastewater having a high level of HCO_3 and low level
34 of $\text{NH}_4\text{-N}$ (ratio of 10:20:1) favored only microalgal growth, and had poor P removal due to a
35 shortage of $\text{NH}_4\text{-N}$ for PAOs growth. However, lowering the HCO_3 level and increasing the $\text{NH}_4\text{-N}$
36 N level (ratio of 10:10:4) balanced PAOs and microalgae symbiosis, and had a positive influence
37 on P removal. Under this mode of operation, the system was able to operate without external
38 aeration and achieved net P removal of $10.33 \pm 1.45 \text{ mg L}^{-1}$ at an influent COD of 100 mg L^{-1} . No
39 significant variation was observed in the reactor performance for different light intensities,
40 suggesting that the system can be operated at low light intensities without affecting P removal.

41

42 **Keywords:** Enhanced biological phosphorus removal; poly-phosphate accumulating organisms;
43 photo activated sludge; microalgae; PAOs-microalgae symbiosis.

44

45 **1. Introduction**

46 Removal of phosphorus (P) from wastewaters before discharge is essential to mitigate negative
47 environmental impacts. This can be achieved either chemically or biologically, although biological
48 removal, via enhanced biological phosphorus removal (EBPR) in activated sludge systems, is

49 effective in achieving higher P removal efficiencies at lower operational costs than chemical
50 alternatives (Bashar et al., 2018). In the conventional EBPR process, mixed liquor is recirculated
51 through alternating anaerobic-aerobic stages to promote the growth of poly-phosphate
52 accumulating organisms (PAOs). In the anaerobic stage, PAOs take up volatile fatty acids (VFAs)
53 present in the influent and store them as poly- β -hydroxyalkanoates (PHA), using glycogen as a
54 reducing agent, and obtain the required energy through the hydrolysis of intracellularly stored poly-
55 phosphates (poly-P) (Wentzel et al., 2008). The hydrolysis of poly-P results in the anaerobic
56 release of ortho-phosphate ($\text{PO}_4\text{-P}$) into the bulk liquid. In the aerobic stage, PAOs oxidize the
57 PHA, obtaining energy for replenishing glycogen for growth and $\text{PO}_4\text{-P}$ uptake (Wentzel et al.,
58 2008). Overall, the oxygen (O_2) required by the EBPR process is usually supplied by mechanical
59 aeration, which may increase the operational costs of aeration, and contribute up to 45-75 % of the
60 total energy costs of activated sludge systems (Rosso et al., 2008).

61 In this context, photosynthetic oxygen generation by microalgae has been utilized for wastewater
62 treatment in the presence of natural or artificial lighting (e.g. wastewater stabilization ponds (WSP)
63 and high rate algal ponds (HRAP); Craggs et al., 2014). However, these systems require a large
64 surface area, and may have poor a nutrient removal capacity when compared to conventional
65 activated sludge systems (Craggs et al., 2003; Mara, 2004; Sutherland et al., 2014). The recent
66 development of photo-activated sludge (PAS) systems at laboratory-scale aims to exploit the
67 synergetic benefits of microalgae and activated sludge systems (Abouhend et al., 2018; Ahmad et
68 al., 2019). In this process, microalgae and bacteria co-exist in flocs or granules in a symbiotic
69 relationship that allows for exchange of carbon dioxide (CO_2) and O_2 . In addition to oxidation of
70 organic matter (OM) and nitrification, microalgal:bacterial flocs can also assimilate nitrogen (N),
71 P, and OM in large quantities for growth (Manser et al., 2016; Rada-Ariza et al., 2017; Ji et al.,

72 2020a). Furthermore, PAS systems at the laboratory-scale have been shown to improve biomass
73 settleability of microalgae, enhance solid-liquid separation (Ji et al., 2020a), and reduce the
74 system's CO₂ footprint (Anbalagan et al., 2017).

75 The development of PAOs in PAS systems may also improve biological P removal
76 (Carvalho et al., 2018). Furthermore, the intracellular poly-P stored by PAOs may increase the
77 density and settleability of the microalgae-bacteria flocs (Schuler et al., 2001). Carvalho et al.
78 (2018) developed a laboratory-scale EBPR-PAS system and attained higher P removal efficiency
79 ($79 \pm 8\%$) without external aeration, at a low influent chemical oxygen demand (COD)/P ratio.
80 However, from the perspective of design parameters, the interaction between PAOs and
81 microalgae is not fully understood (Carvalho et al., 2018). There are significant knowledge gaps
82 regarding the effect of substrate composition and light intensity on EBPR in systems containing a
83 mixture of PAOs and microalgae. Wastewater composition and solar radiation can demonstrate
84 substantial variability, both temporally and spatially (Henze and Comeau, 2008; EUMETSAT,
85 2020), therefore, a better understanding of the influence of these parameters is important for the
86 further development of EBPR-PAS systems.

87 Thus, to address this knowledge gap, a stoichiometric EBPR-PAS model-based design was first
88 investigated to understand the impact of carbon (C) and N on P removal. Following the model
89 development, two different synthetic wastewater compositions of COD:HCO₃:NH₄-N of 10:20:1
90 and 10:10:4 were examined in controlled laboratory reactor experiments to evaluate P removal. In
91 addition, the short-term effects of four incident light intensities (350, 262.5, 175, and 87.5 μmol
92 $\text{m}^{-2} \text{sec}^{-1}$), were also investigated to evaluate the stability of symbiotic PAOs-microalgal capacity
93 for P removal.

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96 **2. Materials and Methods**

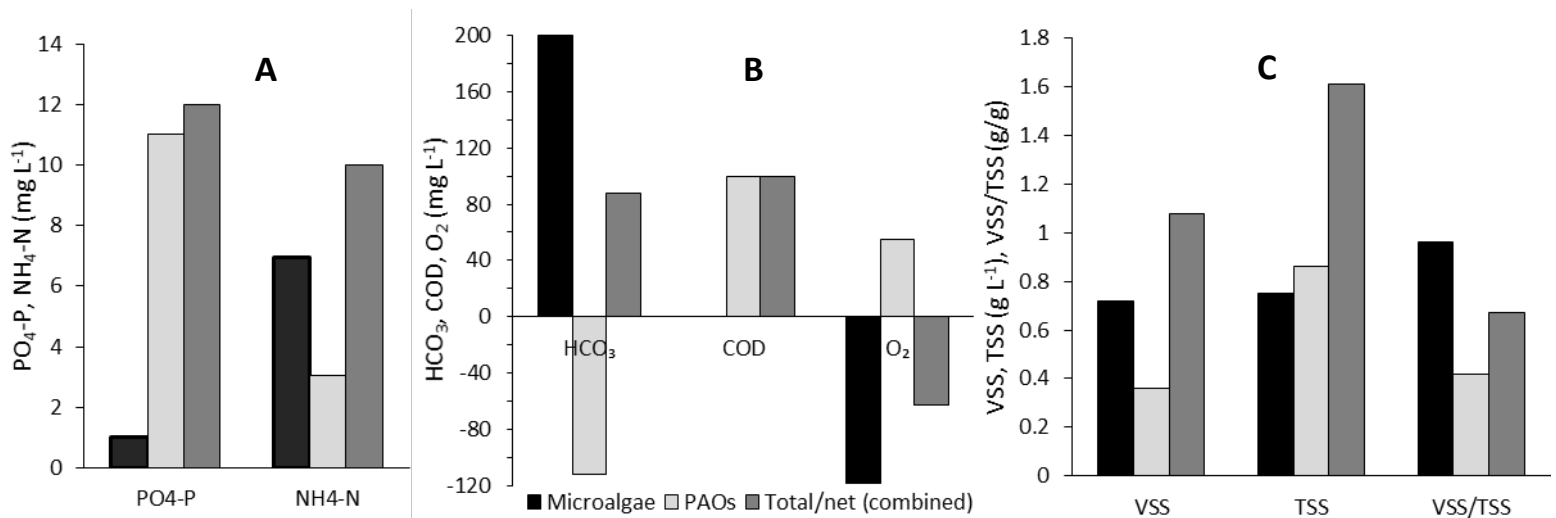
97 **2.1 Model-based design**

98 Prior to the start-up, a model-based design approach was used to identify the theoretically optimal
99 operating conditions (including synthetic wastewater composition, light intensity, and operational
100 and environmental conditions). The model (Tables A.1 to A.3, Appendix A of the Supplementary
101 Information) was developed in MS Excel™ using the EBPR stoichiometric-based, steady-state
102 design model developed by Comeau et al. (1986), Wentzel et al. (1990), and Smolders et al.
103 (1994a,b). This model was modified and extended by coupling the oxygen requirements of the
104 aerobic EBPR activity to the oxygen generation by microalgae (Mara, 2004).

105 To develop the model, the following assumptions were made: a) operation of an EBPR-PAS
106 system in a completely mixed sequencing batch reactor (SBR) (Wilderer et al., 2001) under
107 alternating anaerobic-aerobic EBPR conditions, with dark conditions during the anaerobic stage
108 and illuminated conditions during the aerobic stage; b) operation of the EBPR-PAS system with a
109 total suspended solids (TSS) concentration of 1-2 g L⁻¹ to avoid light hindrance, by controlling
110 sludge retention time (SRT) (Arashiro et al., 2017) and the synthetic feed; c) avoidance of the
111 presence of nitrate in the anaerobic stage by suppressing the growth of nitrifying organisms in the
112 EBPR-PAS system by applying a minimum net aerobic SRT of 3 days (Brdjanovic et al., 1998)
113 and through the addition of a nitrification inhibitor; d) avoidance of P-limiting conditions for PAOs
114 and microalgae by taking into account the maximum poly-P storage of PAOs ($f_{p,vss\ PAOs}$) (38%
115 of volatile suspended solids (VSS); Wentzel et al., 1990) and the minimum requirements to cover
116 the microalgal growth requirements ($f_{p,vss\ algae}$) (1.3% VSS; Mara, 2004); e) supplying enough
117 ammonium-N (NH₄-N) in the feed for PAOs (10% VSS; Henze et al. 2008) and microalgae (9.2%

118 VSS; Mara, 2004); f) adjusting the inorganic carbon concentration in the feed taking into account
119 the CO₂ generation of PAOs so that microalgae should be the only source of oxygen for PAOs
120 when implementing the illuminated stage; and g) light requirement was estimated taking into
121 account light penetration/attenuation as a function of the TSS concentration using the Lambert-
122 Beer equation (Swinehart, 1962) (Appendix B).

123 Fig. 1 shows the summary results of the model. For balanced PAOs-microalgae symbiosis, the
124 model suggested the composition of the synthetic wastewater should contain 100 mg L⁻¹ of COD,
125 12 mg L⁻¹ of PO₄-P, 88 mg L⁻¹ of HCO₃, and 10 mg L⁻¹ of NH₄-N (Fig. 1-a,b). Under these
126 conditions, the overall VSS and TSS concentrations were estimated at 1.08 g L⁻¹ and 1.6 g L⁻¹ (Fig.
127 1-c), respectively, which were within the limits specified in the assumptions. Moreover, the oxygen
128 produced by microalgae would be double the PAOs requirements (Fig. 1-b). Based on the
129 prediction of the model, these concentrations were applied to the start-up phase with only minor
130 modifications: the inorganic carbon was 200 mg HCO₃ L⁻¹ instead of 88 mg HCO₃ L⁻¹ (Fig. 1-b),
131 which supplied the total inorganic carbon required, not assuming that PAOs would retrieve the
132 theoretically calculated 112 mg HCO₃ L⁻¹ (Fig. 1-b) as CO₂ anaerobically and aerobically
133 according to Smolders et al. (1994a,b). The model estimated an incident light intensity of 145
134 μmol m⁻² sec⁻¹ (Table A.3, Appendix A) for the photosynthesis process. However, a light intensity
135 of 350 μmol m⁻² sec⁻¹ was used in the start-up phase to avoid light limitation for microalgal growth.



136 **Fig. 1** Model results for: a) influent concentrations of PO₄-P and NH₄-N; b) concentrations of
 137 HCO₃⁻, COD, and O₂ (positive values mean consumption/uptake, and negative values mean
 138 production/release); and c) the relevant generated biomass (VSS, TSS, VSS/TSS) for microalgae,
 139 PAOs and combination.

140

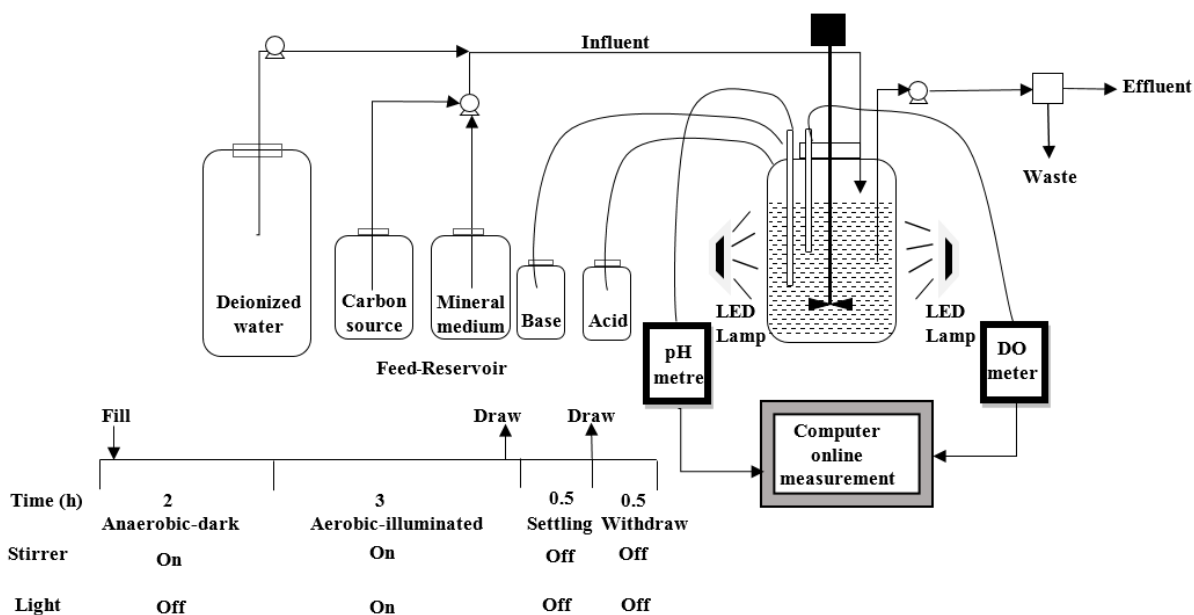
141 2.2 Reactor configuration and enrichment of the EBPR-PAS system

142 Two cylindrical double-jacketed glass reactors, each with an internal diameter of 12.5 cm and an
 143 active volume of 2.5 L, were used in the study. Reactor One (R1; Fig. 2) was the main reactor and
 144 used for the enrichment and development of the PAOs-microalgae consortia to test the synergetic
 145 effect of microalgae and PAOs to perform EBPR (referred to as an EBPR-PAS system). Reactor
 146 Two (R2), the study control, was operated as a conventional EBPR (without microalgae and light)
 147 (referred to as an EBPR system). R1 was inoculated with 600 ml of activated sludge from a
 148 wastewater treatment plant (WWTP) (Harnaspolder, Den Horn, The Netherlands) with a TSS
 149 concentration of 6 g L⁻¹. The activated sludge was mixed with 600 ml of five species of
 150 microalgae/cyanobacteria (120 ml each; *Scenedesmus quadricauda*, *Anabaena variabilis*,
 151 *Chlorella sp.*, *Chlorococcus sp.*, *Spirulina sp*) with an average concentration of 1 g TSS L⁻¹, similar
 152 to those used by van der Steen et al. (2015). This led to a total initial TSS concentration of 1.75 g
 153 L⁻¹ in R1. R2 was inoculated with an enriched culture of PAOs from an EBPR reactor (Welles et

154 al., 2017). Due to operational challenges with R1, which showed poor EBPR activities in the start-
155 up phase, 25 to 30 ml of enhanced PAOs culture from R2 was seeded on daily basis to the
156 microalgae biomass in R1 from day 25 to 36 and day 41.

157 The EBPR-PAS system (R1) was operated under alternating anaerobic-aerobic EBPR conditions,
158 with dark conditions during the anaerobic stage and illuminated conditions during the aerobic
159 stage, to enhance the growth of microalgae. To achieve this, R1 was run as an SBR with four
160 cycles of 6 h per day, comprising a 2 h dark/anaerobic stage, a 3 h illuminated/aerobic stage, a 0.5
161 h settling stage, and a 0.5 h effluent withdrawal stage (Fig. 2). An ADI controller and BioXpert
162 software (Applikon, Delft, The Netherlands) were used to operate the SBR and for data acquisition.
163 Mixing took place in the dark/anaerobic and illuminated/aerobic stages at 500 rpm (van Loosdrecht
164 et al., 2016). The temperature was maintained at 20 ± 1 °C throughout all stages by a LAUDA
165 system (Lauda-Königshofen, Germany). The pH was maintained at 7.5 ± 0.1 to favor PAOs over
166 glycogen-accumulating organisms (GAO; Lopez-Vazquez et al., 2009), through the automatic
167 dosing of either 0.4 M HCl or 0.4 M NaOH. For illumination, eight light-emitting-diode (LED)
168 lamps (40W, Phillips, The Netherlands) were used, with four of each located at opposite sides of
169 the reactor. During each cycle, from min 5 to min 10 of the anaerobic stage, synthetic wastewater
170 was pumped from the influent feeding tank to R1 using a peristaltic pump, while a second
171 peristaltic pump, positioned in R1, served two purposes: to withdraw 105 ml of the mixed liquor
172 for the last 5 mins of the aerobic stage, thereby controlling the SRT to 6 days, and to discharge the
173 effluent during the withdrawal stage (Fig. 2). During the effluent withdrawal stage, half of the
174 working volume was removed, so as to attain an hydraulic retention time (HRT) of 12 h. During
175 the start-up phase (P1), an air compressor, controlled by an on-off valve, supplied the oxygen to
176 the reactor during the aerobic period at a point not exceeding 20% of saturation (around 1.8 mg L⁻¹

177 1). The air compressor was not used when the system was solely dependent on the microalgae
 178 oxygen in the phases following the start-up phase. Nitrogen gas (N₂) was sparged into the reactor
 179 during the first 25 mins of the anaerobic stage to generate anaerobic conditions.



182 **Fig. 2** Main reactor set-up (R1)

184 R2 contained the same medium and had the same operational conditions as in R1 (SRT: 6 days,
 185 HRT: 12 h). However, oxygen was supplied to R2 during all the cycles of the aerobic stages,
 186 whereas in R1, because of the reliance on microalgae to provide oxygen, external aeration was
 187 supplied only in the start-up phase (P1) when the dissolved oxygen (DO) saturation dropped below
 188 20%. In addition, R2 was not exposed to alternating dark and illumination conditions as was the
 189 case with R1.

190 2.3 Experimental phases and synthetic medium

191 Table 1 shows the operating conditions for the EBPR-PAS system, which were determined based
 192 on the outcomes of the model-based design. It comprised three experimental phases, with a total
 193 duration of 100 days. In phase 1 (P1), the EBPR-PAS system was loaded with synthetic wastewater

194 [COD:100 mg L⁻¹; P:12 mg L⁻¹; COD:HCO₃:NH₄-N of 10:20:1 mg:mg:mg]. After ensuring that
 195 the microalgae were growing well enough to produce sufficient oxygen (DO saturation level >
 196 20%), the external aeration supply was gradually reduced over 11 days until the EBPR-PAS system
 197 operated without external aeration. In phase 2 (P2), in order to optimize the nutrients and avoid
 198 limiting growth conditions for both organisms, the NH₄-N concentration was increased by a factor
 199 of four and the inorganic carbon reduced by half (COD:HCO₃:NH₄-N of 10:10:4 mg:mg:mg), and
 200 the system was allowed to stabilize. When no significant changes in the effluent parameters were
 201 observed (for at least 3*SRT= 18 days), the system was assumed to have reached pseudo-steady-
 202 state conditions. In phase 3 (P3), the short-term effects of different incident light intensities (350,
 203 262.5, 175, and 87.5 μmol m⁻² sec⁻¹), each lasting three days, were investigated on the last day for
 204 each light intensity. These intensities were within the range reported by EUMETSAT (2020) in
 205 Northern European conditions. This phase aimed to study the effect of light intensity on DO
 206 generation, and evaluate the culture response to light fluctuations and, therefore, the performance
 207 of the EBPR-PAS system.

208 **Table 1** Operational conditions of the main reactor (R1) during the study period of 100 days.

<i>Phase</i>	<i>SRT (days)</i>	<i>HRT (hrs)</i>	<i>External aeration</i>	<i>Light intensity μmol m⁻² sec⁻¹</i>	<i>COD:HCO₃: NH₄-N</i>	<i>Seeding of PAOs from R2</i>	<i>Operational days</i>
P1	6	12	+/-	350	10:20:1	+++	40
P2	6	12	--	350	10:10:4	+/--	40
P3	6	12	--	350, 262.5, 175, 87.5	10:10:4	---	20

(+) with external aeration or PAOs seeding (-) without external aeration or PAOs seeding

209

210 The influent wastewater comprised a carbon source, a mineral medium, and deionized water, each
 211 contained in separate containers (Fig. 2). The carbon source consisted of 3:1 acetate: propionate.
 212 This COD ratio favors the growth of PAOs over GAO (Lopez-Vazquez et al., 2009). The carbon

213 source and mineral medium were autoclaved for one hour at 115 °C before use. The final
214 composition of wastewater in the influent consisted of: 160 mg L⁻¹ of NaAc.3H₂O (2.36 C-mmol
215 L⁻¹, 75 mg COD L⁻¹), 0.0167 ml of C₃H₆O₂ (0.68 C-mmol L⁻¹, 25 mg COD L⁻¹), 38 mg L⁻¹ of
216 NH₄Cl (0.715 N-mmol L⁻¹, 10 mg N L⁻¹) (increased to 152 mg L⁻¹ (2.86 N-mmol L⁻¹, 40 mg N L⁻¹
217 ¹) in P2 and P3), 48 mg L⁻¹ of NaH₂PO₄ (0.4 P-mmol L⁻¹, 12 mg P L⁻¹), 280 mg L⁻¹ of NaHCO₃
218 (3.34 C-mmol L⁻¹, 200 mg HCO₃ L⁻¹) (reduced to 140 mg L⁻¹ (1.67 C-mmol L⁻¹, 100 mg HCO₃ L⁻¹
219 ¹) in P2 and P3), 75 mg L⁻¹ of MgSO₄.7H₂O, 36 mg L⁻¹ of CaCl₂.2H₂O, 16.0 mg L⁻¹ of KCl, 3.4
220 mg L⁻¹ of FeSO₄.7H₂O, 10 mg L⁻¹ of EDTA.Na₂, 2 mg L⁻¹ of allyl-N-thiourea (ATU) to inhibit
221 nitrification, 1 mg L⁻¹ of yeast extract, and trace elements as described in Becker (1994) for
222 microalgae growth. The trace elements receipt also attained PAOs requirements as described by
223 Smolders et al. (1994a,b). Influent concentrations were diluted after mixing with the half reactor
224 volume remaining from the previous cycle.

225 **2.4 Analysis**

226 Acetate and propionate were measured using Varian 430-GC Gas Chromatography (Varian BV,
227 The Netherlands) equipped with a split injector (200 °C), a WCOT Fused Silica column (105 °C)
228 and coupled to a FID detector (300 °C). Helium gas was used as the carrier gas and 50 mL of
229 butyric acid as the internal standard. Ammonium was measured using spectrophotometric methods
230 as described in NEN 6472 (1983). Phosphate was measured using the ascorbic acid
231 spectrophotometric method as described in APHA (2005). Samples that were not analyzed
232 immediately were preserved at 4 °C for NH₄-N and PO₄-P measurements and -20 °C for VFA
233 measurements for a maximum of one week before analysis. Total inorganic carbon (TIC) was
234 measured using a TOC-L analyzer equipped with an ASI-L autosampler (Shimadzu, Kyoto,
235 Japan). Total suspended solids and VSS were measured using gravimetric techniques (APHA,

236 2005). Light intensity was measured using a light meter Li-250 (Li-COR, United States).
237 Chlorophyll-*a* was measured using the ethanol extraction Spectrophotometric method, described
238 in the Dutch standard method (NEN 6520, 1982). An Avantium Crystalline PV (Crystalline
239 analyzer) was used to investigate particle size distribution of the flocs. For the identification of
240 microalgae/cyanobacteria species based on morphology, an advanced optical microscope Olympus
241 BX53 (Shinjuku, Tokyo, Japan) was used. To enhance the visualization of the mixed culture cells,
242 DAPI staining was applied to record prokaryotic/bacteria and eukaryotic/microalgae DNA through
243 fluorescence (Nielsen et al., 2009). The pictures were then captured with an Olympus BX51
244 (Shinjuku, Tokyo, Japan).

245

246 **2.5 Calculations**

247 **2.5.1. Sludge volume index**

248 To calculate the sludge volume index (SVI) (Eq. 1), the mixed culture was poured into a 2 L
249 capacity vertical cylinder and allowed to settle. The settled sludge volume was measured after 30
250 mins, and the TSS concentration of the mixed culture was measured gravimetrically (APHA,
251 2005).

$$252 \quad SVI \text{ (ml g}^{-1}\text{)} = \frac{\textit{Settled sludge volume (ml L}^{-1}\text{)} \times 1000}{\textit{Suspended solids (mg L}^{-1}\text{)}} \quad \text{Eq. (1)}$$

253 **2.5.2. Kinetic and stoichiometric parameters**

254 The kinetic profiles of NH₄-N, PO₄-P, VFAs, TIC, and DO in P1-P3 were monitored to assess the
255 performance of the EBPR-PAS system and were also used as metrics to adjust the medium
256 composition in P2.

257 The maximum P release rate (in mg PO₄-P L⁻¹ h⁻¹) was calculated from the slope of the graph by
258 adjusting the linear regression line to the experimental concentrations determined along the highest
259 P release period (start of dark/anaerobic stage). Phosphorus release (in mg PO₄-P L⁻¹) was
260 calculated as the difference between P concentrations at the start and end of the dark-anaerobic
261 stage (Eq. 2).

$$262 \quad P_{\text{release}} = P_{\text{end of anaerobic}} - P_{\text{start of anaerobic}} \quad (\text{Eq. 2})$$

263 The maximum P uptake rate (mg PO₄-P L⁻¹ h⁻¹) was calculated from the slope of the graph by
264 adjusting the linear regression line to the experimental concentrations determined during the
265 highest P uptake period (start of illuminated/aerobic stage). Total P uptake (in mg PO₄-P L⁻¹) was
266 calculated as the difference between P concentrations at the start and end of the illuminated/aerobic
267 stage (Eq. 3).

$$268 \quad P_{\text{uptake total}} = P_{\text{start of aerobic}} - P_{\text{end of aerobic}} \quad \text{Eq. (3)}$$

269 The net P removal (in mg PO₄-P L⁻¹) was calculated as the difference between P concentrations of
270 the influent and at the end of a 6 h cycle (Eq. 4).

$$271 \quad P_{\text{net-removal}} = P_{\text{influent}} - P_{\text{effluent}} \quad \text{Eq. (4)}$$

272 The maximum VFA uptake rate (in mg COD L⁻¹ h⁻¹) was calculated from the slope of the graph
273 by adjusting the linear regression line to the experimental concentrations determined along the
274 highest VFA uptake period (start of dark/anaerobic stage). VFA consumed (in mg COD L⁻¹) was
275 calculated as the difference between COD concentrations at the start and end of the dark/anaerobic
276 stage (Eq. 5).

$$277 \quad VFA_{\text{consumed}} = VFA_{\text{start of anaerobic}} - VFA_{\text{end of anaerobic}} \quad \text{Eq. (5)}$$

278 Phosphorus release/VFA consumed was calculated and presented as P-mmol/C-mmol. The NH₄-
279 N uptake rate (in mg NH₄-N L⁻¹ h⁻¹) was calculated from the slope of the graph by adjusting the
280 linear regression line to the experimental concentrations determined along the different periods of
281 the illuminated/aerobic stage. Statistical analysis was performed using the t-test (two-tailed) using
282 Excel. The standard error (SE) was calculated using Eq. (7):

$$283 \quad SE = \text{STDEV} / \sqrt{N} \text{ (where N is the number of samples).} \quad \text{Eq. (6)}$$

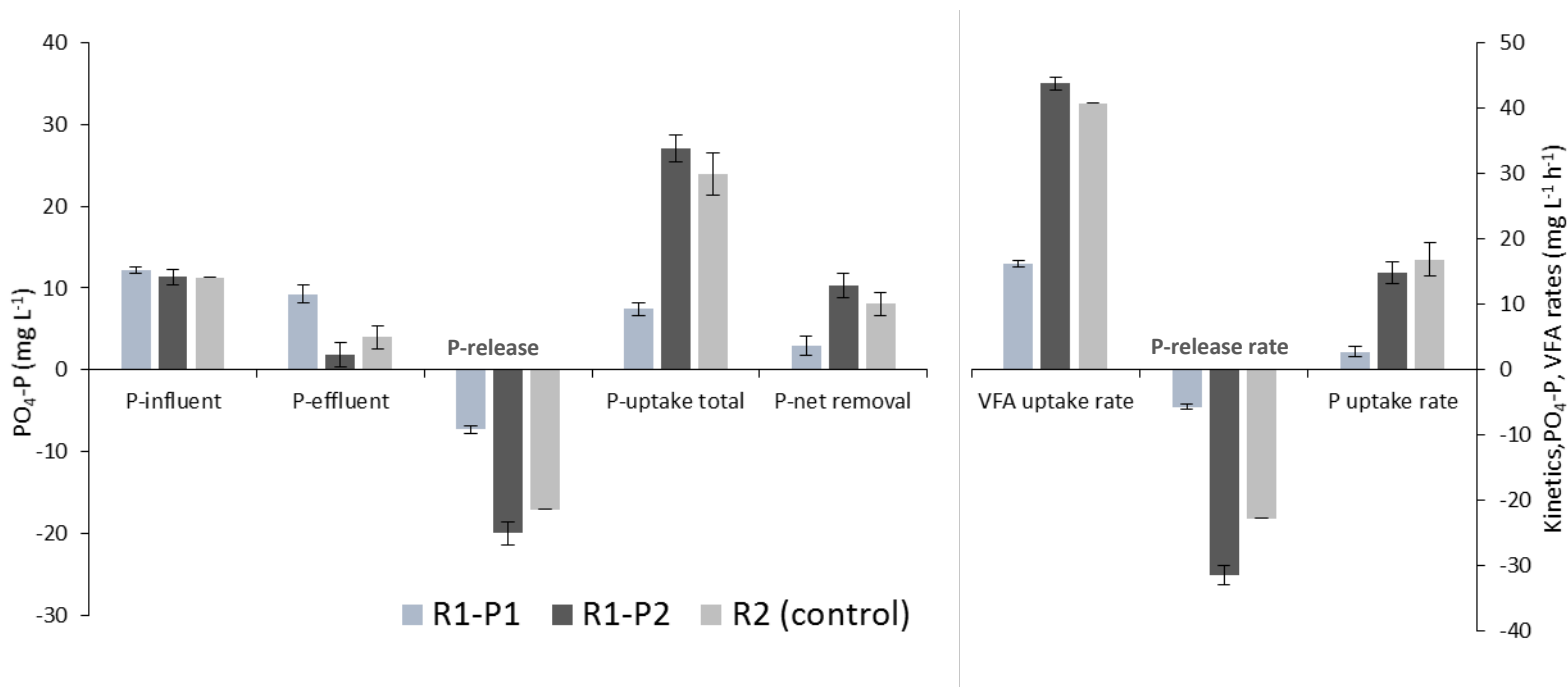
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285 **3. Results and discussion**

286 **3.1 First Experimental phase (COD:HCO₃:NH₄-N of 10:20:1)**

287 Phase 1 (P1) was the starting point of the EBPR-PAS cultivation system based on the steady-state
288 model prediction (COD:HCO₃:NH₄-N: 10:20:1). The EBPR-PAS system (R1) displayed
289 excellent algal/photosynthesis activities as measured by the utilization of inorganic carbon and the
290 production of oxygen, which occasionally exceeded 300% of the saturated DO. However, R1
291 demonstrated poor P release and uptake (Fig. 3). The P release and total P uptake for R1 in P1
292 were $7.3 \pm 0.48 \text{ mg L}^{-1}$ (SE= 0.24, n= 4) and $7.2 \pm 2.8 \text{ mg L}^{-1}$ (SE= 1.05, n= 7), respectively, (Fig.
293 3). Overall, R1 had a final effluent P concentration of $9.45 \pm 1.05 \text{ mg L}^{-1}$ (SE= 0.25, n= 9), with a
294 net P removal of only $2.75 \pm 1.05 \text{ mg L}^{-1}$ (SE= 0.25, n= 9; Fig. 3). In contrast, the conventional
295 EBPR system, R2 (control), demonstrated good P release and total P uptake (Fig. 3). The average
296 P release and total P uptake were $17.1 \pm 1.3 \text{ mg L}^{-1}$ and $24 \pm 2.5 \text{ mg L}^{-1}$ (SE= 1.04, n= 6),
297 respectively, (Fig. 3). The average P concentration in the final effluent for R2 was $4.0 \pm 1.4 \text{ mg L}^{-1}$
298 (SE= 0.41, n= 12), with a net removal of $8.01 \pm 1.38 \text{ mg P L}^{-1}$ (SE= 0.4, n= 12) (Fig. 3).
299 Kinetically, R2 performed better than R1 in P1 (Fig. 3). The anaerobic consumption rate of VFA
300 by PAOs for R2 ($40.2 \text{ mg L}^{-1} \text{ h}^{-1}$) was 2.5 times higher than R1 ($16 \text{ mg L}^{-1} \text{ h}^{-1}$). The P release rate

301 during the anaerobic stage for R2 ($23 \text{ mg L}^{-1} \text{ h}^{-1}$) was four times higher than R1 ($5.7 \text{ mg L}^{-1} \text{ h}^{-1}$;
 302 Fig. 3). The maximum P uptake rate during the aerobic period for R2 ($16.87 \text{ mg L}^{-1} \text{ h}^{-1}$) was almost
 303 six times higher than in R1 ($2.76 \text{ mg L}^{-1} \text{ h}^{-1}$) (Fig. 3)



304 **Fig. 3** Phosphorus and VFA parameters for the EBPR-PAS system (R1) during P1 and P2, and the
 305 control reactor (conventional EBPR system, R2)
 306

307 To improve R1 performance, 25 to 30 ml of enhanced PAOs culture ($VSS = 0.4 \pm 0.03 \text{ g L}^{-1}$, $TSS =$
 308 $0.7 \pm 0.06 \text{ g L}^{-1}$) from R2 was seeded regularly to the microalgae biomass in R1 from day 25 to 36.
 309 Despite this addition, R1 did not demonstrate any capacity for P removal during P1, although both
 310 R1 & R2 were supplied with the same medium and operated under the same conditions. The poor
 311 growth of PAOs culture in R2 may have been due to different stresses in the cultivation system
 312 (e.g. O_2 saturation, nutrient stress (competition for NH_4-N), micro-algal concentration, sludge age).
 313 Therefore, the interplay between PAOs and microalgae was not balanced, with microalgae
 314 dominating P1.

315 To investigate the cause of the poor P removal in R1, kinetic studies were performed for R1 (on
316 day 24) and R2 (on day 33), and compared to each other (Fig. 4a-c). The results showed that only
317 20 mg L⁻¹ of COD was consumed during the anaerobic stage, and more than half the influent COD
318 (30 mg L⁻¹) escaped into the aerobic stage for R1 (Fig. 4a). On the other hand, the influent COD
319 was fully consumed and stored by PAOs in the anaerobic stage for R2 (Fig. 4a), which was an
320 indication of good EBPR activity. The P profile in Fig. 4b shows both P release and P uptake were
321 very low in R1 compared to R2: the maximum P release rate during the anaerobic stage for R2 (23
322 mg L⁻¹ h⁻¹) was eight times higher than R1 (3 mg L⁻¹ h⁻¹; Fig. 4b). The maximum P uptake rate
323 during the aerobic period for R2 (16.87 mg L⁻¹ h⁻¹) was almost six times higher than in R1 (2.72
324 mg L⁻¹ h⁻¹; Fig. 4b). The NH₄-N profile in Fig. 4c shows that NH₄-N uptake in R1 started from the
325 beginning of the aerobic stage, with a rate of 3.37 mg NH₄-N L⁻¹ h⁻¹, and was almost fully
326 consumed within the first 45 mins. In R2, there was a 30 minute lag in the aerobic stage before
327 PAOs started to uptake NH₄, and then uptake occurred at 0.44 mg-NH₄-N L⁻¹ h⁻¹, a lower rate than
328 observed in R1 (Fig. 4c). As a result of the higher NH₄-N uptake in R1, PAOs growth in this
329 system was likely inhibited by a shortage of N, although the total demand of NH₄-N by PAOs was
330 very low according to R2 as shown in Fig. 4c. Thus, ammonium was likely taken up by microalgae
331 and potentially by other organisms rather than PAOs in R1. Moreover, the loss of NH₄ by
332 nitrification was not expected due to the addition of the nitrification inhibitor, ATU.

333

334

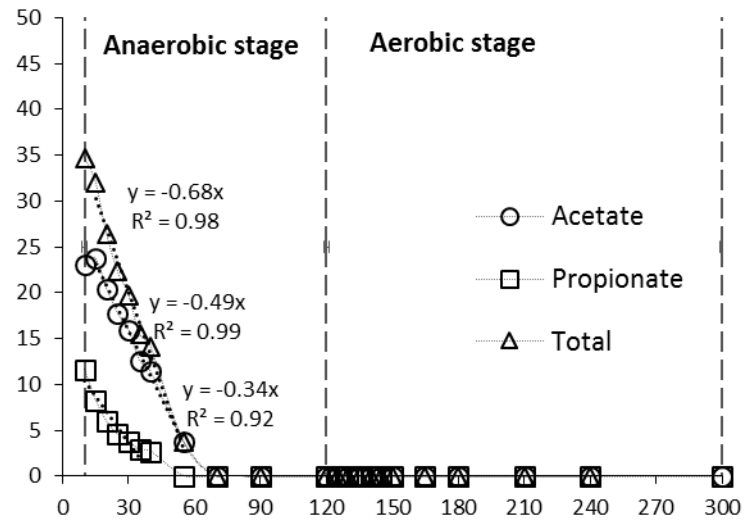
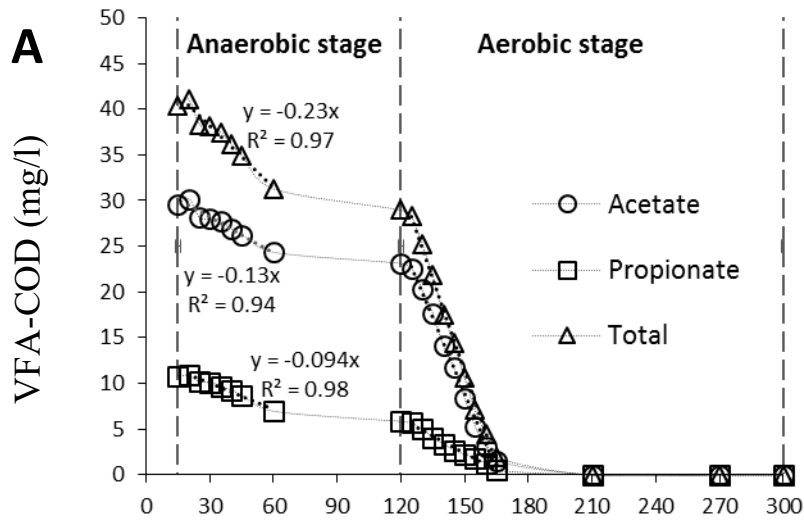
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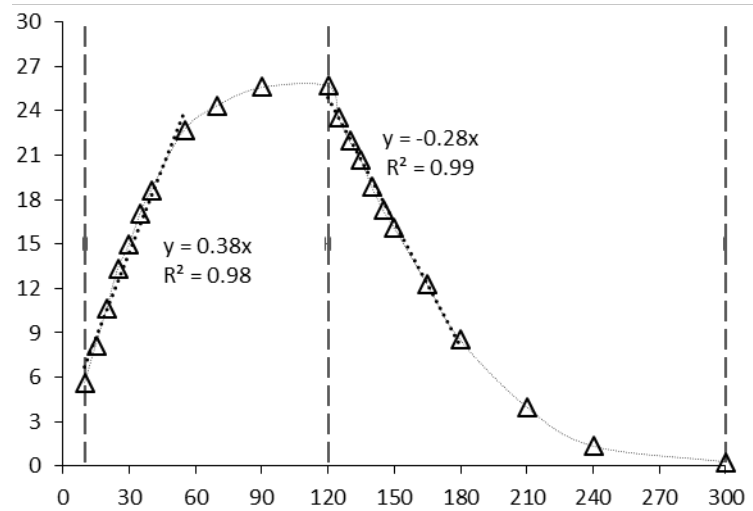
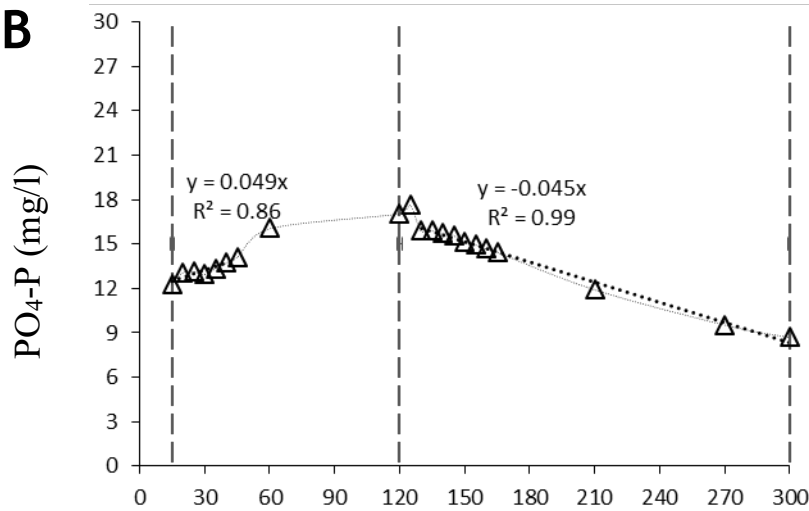
EBPR-PAS (R1-P1)

EBPR (R2)

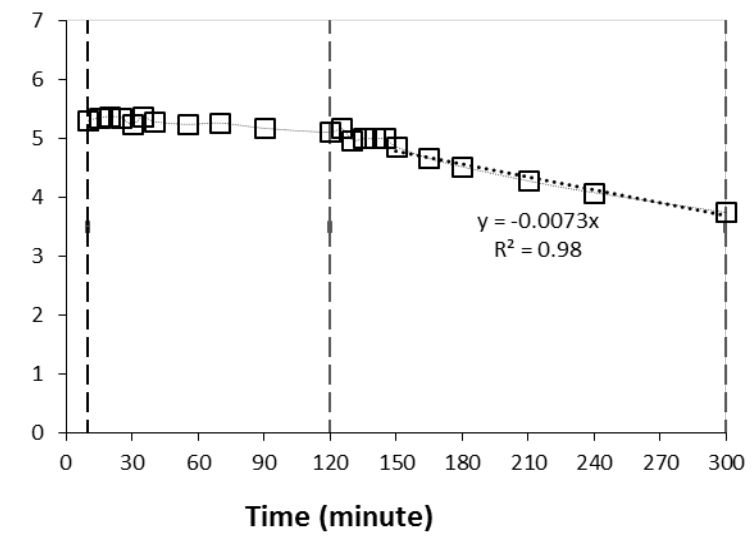
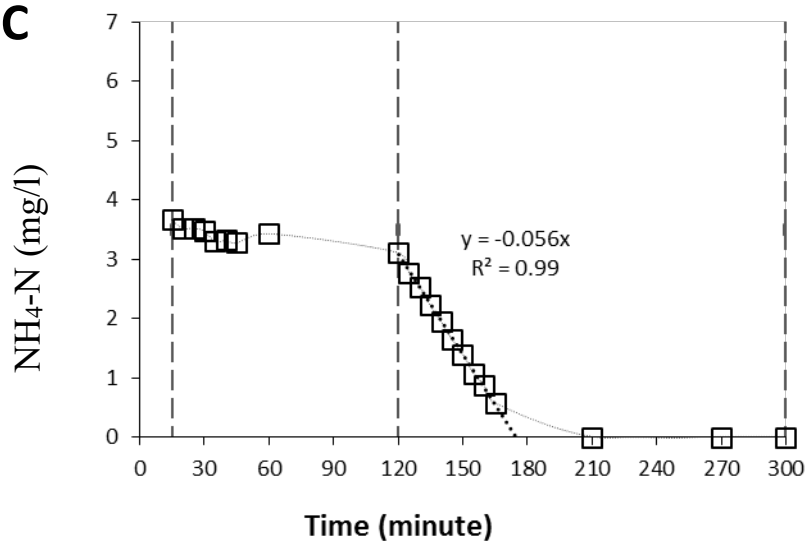
A



B



C



337 **Fig. 4** Kinetic studies for R1 during P1 on day 24 of operation (left) and R2 on day 33 of
 338 operation (right); A): VFAs profile; B) phosphate profile; C) ammonium-N profile.

339 Therefore, the $\text{NH}_4\text{-N}$ concentration in the influent of R1 was increased from 10 mg L^{-1} to 40 mg
340 L^{-1} to meet PAOs requirements. Also, the inorganic carbon concentration in the influent was
341 decreased from $200 \text{ mg HCO}_3 \text{ L}^{-1}$ to $100 \text{ mg HCO}_3 \text{ L}^{-1}$, to limit the excess $\text{NH}_4\text{-N}$ uptake by
342 microalgae. Despite this, the new value of the inorganic carbon met the model requirement (88 mg
343 $\text{HCO}_3 \text{ L}^{-1}$; Fig. 1-b), because the initial concentration of inorganic carbon that had been added in
344 P1 did not consider the inorganic carbon produced by PAOs (Section 2.1).

345 **3.2 Second experimental phase (COD:HCO₃:NH₄-N of 10:10:4)**

346 Effective conditions were established by adjusting the nutrient composition (COD, HCO_3 , and
347 $\text{NH}_4\text{-N}$) to a ratio of (10:10:4) according to the results of the kinetic studies in P1. Both P release
348 and P uptake within R1 improved significantly ($P < 0.0001$) during P2, and the results were
349 comparable to the control reactor (R2; Fig. 3). The average P release and total P uptake by R1
350 were $20 \pm 1.43 \text{ mg L}^{-1} \text{ mg L}^{-1}$ (SE= 0.5, n= 8) and $27.8 \pm 1.8 \text{ mg L}^{-1}$ (SE= 0.48, n= 14), respectively,
351 (Fig. 3). The average P concentration in the final effluent for R2 was $1.87 \pm 1.45 \text{ mg L}^{-1}$ (SE=
352 0.25, n= 34), with a net removal of $10.33 \pm 1.45 \text{ mg P L}^{-1}$ (SE= 0.25, n= 34; Fig. 3).

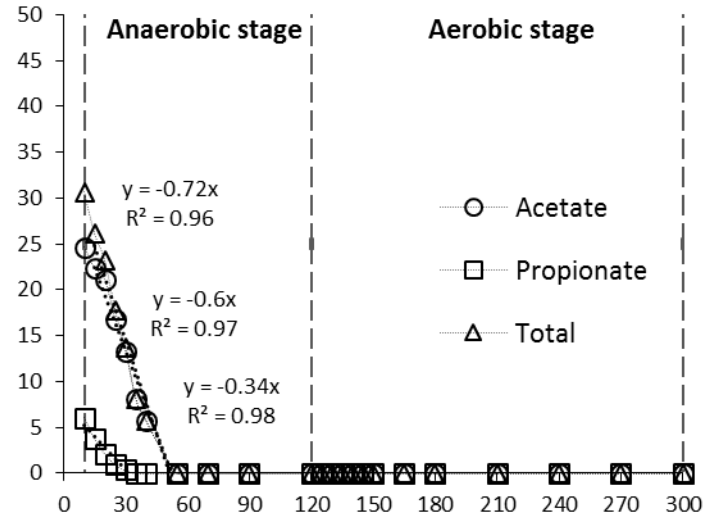
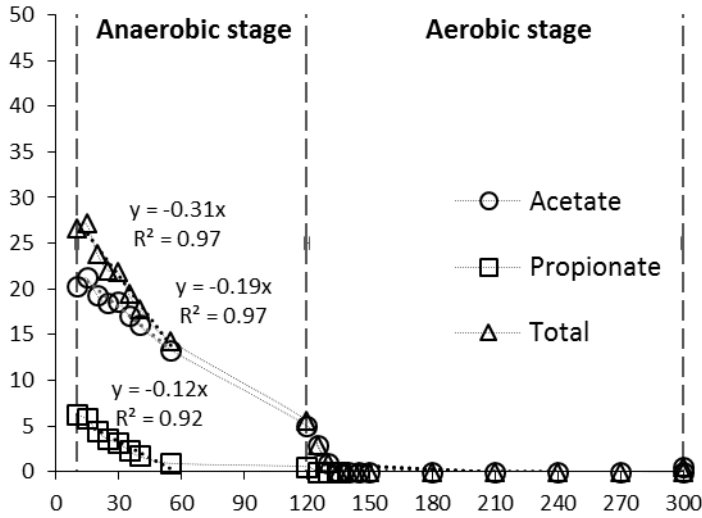
353 The total COD was fully consumed during the first hour of the anaerobic stage at a rate of 40 mg
354 $\text{L}^{-1} \text{ h}^{-1}$ (Fig. 5a), a rate that was similar to previous measurements for R2 (Fig. 4a), and almost three
355 times higher than that achieved in P1 for R1 (Fig. 4a, Fig. 5a). Correspondingly, the P release rate
356 for R1 increased three-fold from $8.4 \text{ mg L}^{-1} \text{ h}^{-1}$ in P1 to $28.2 \text{ mg L}^{-1} \text{ h}^{-1}$ in P2 (Fig. 5b), and P
357 uptake rate for R1 increased six-fold from $2.82 \text{ mg L}^{-1} \text{ h}^{-1}$ in P1 to $16.8 \text{ mg L}^{-1} \text{ h}^{-1}$ in P2 (Fig. 5b),
358 which was similar to R2 (Fig. 4b). Fig. 5c shows there was a lot of ammonium left in the effluent
359 in P2, and the uptake rate was reduced from $3.84 \text{ mg L}^{-1} \text{ h}^{-1}$ in P1 to nearly half in P2 (2.3 mg L^{-1}
360 h^{-1}) (Fig. 5c). This decrease was possibly due to the reduction in microalgal biomass

EBPR-PAS (R1-P1)

EBPR-PAS (R1-P2)

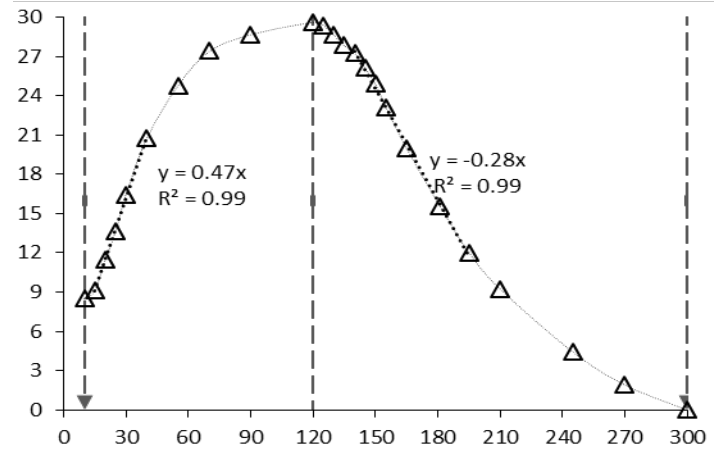
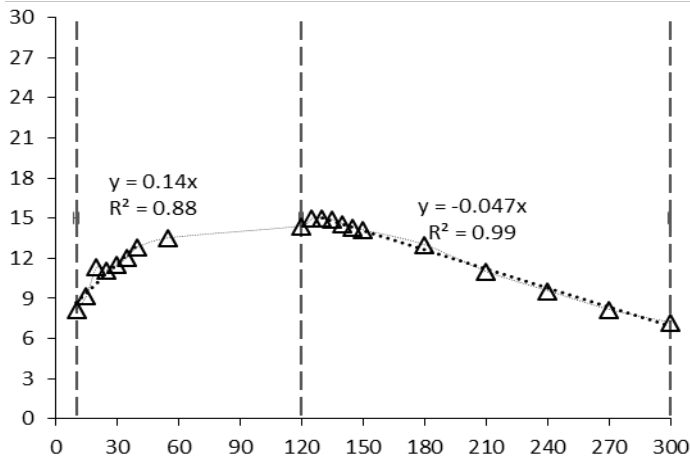
A

VFA-COD (mg L⁻¹)



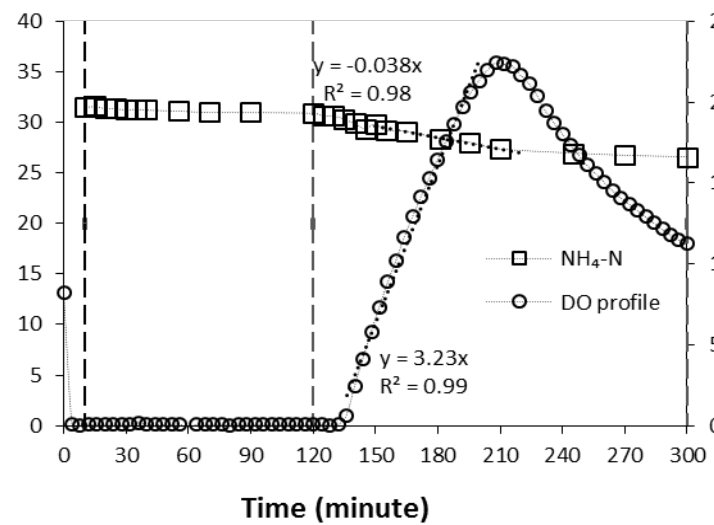
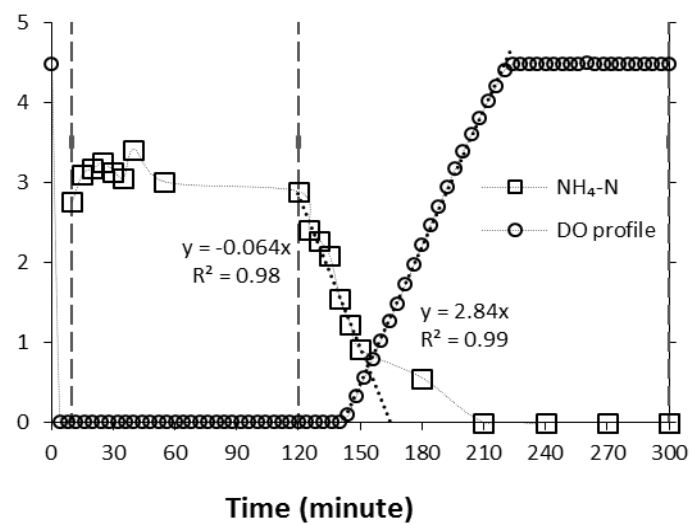
B

PO₄-P (mg L⁻¹)



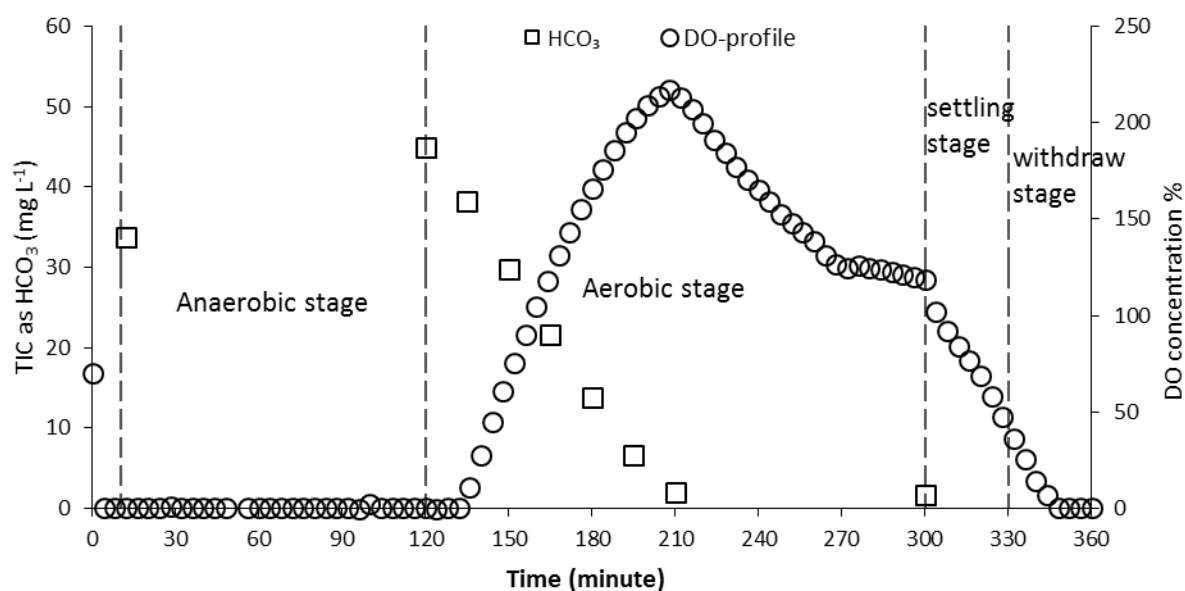
C

NH₄-N (mg L⁻¹),
DO concentration (%)



361 **Fig. 5** Kinetic studies for R1 during P1 on day 26 of operation (left) and P2 on day 64 of
 362 operation (right); a): VFAs profile; b) phosphate profile; c) ammonium profile combined with
 363 DO profile.

364 as a result of reducing influent inorganic carbon. However, the $\text{NH}_4\text{-N}$ uptake rate of R1 in P2 was
 365 still higher than R2 (Fig. 4c), because microalgae and PAOs will co-contribute to the uptake rate
 366 of $\text{NH}_4\text{-N}$ in R1. The total $\text{NH}_4\text{-N}$ uptake in P2 was 4.9 mg L^{-1} (Fig. 5c; 9.8 mg L^{-1} as an influent
 367 concentration before dilution). This concentration was similar to the $\text{NH}_4\text{-N}$ supplied in P1.
 368 Therefore, inorganic carbon reduction was a key adjustment to limit microalgal growth and
 369 cultivate a successful symbiotic relationship between microalgae and PAOs.



370
 371 **Fig. 6** Kinetic study of total inorganic carbon (as HCO_3^-) combined with the DO online profile for
 372 R1 in P2 on day 49 of operation.

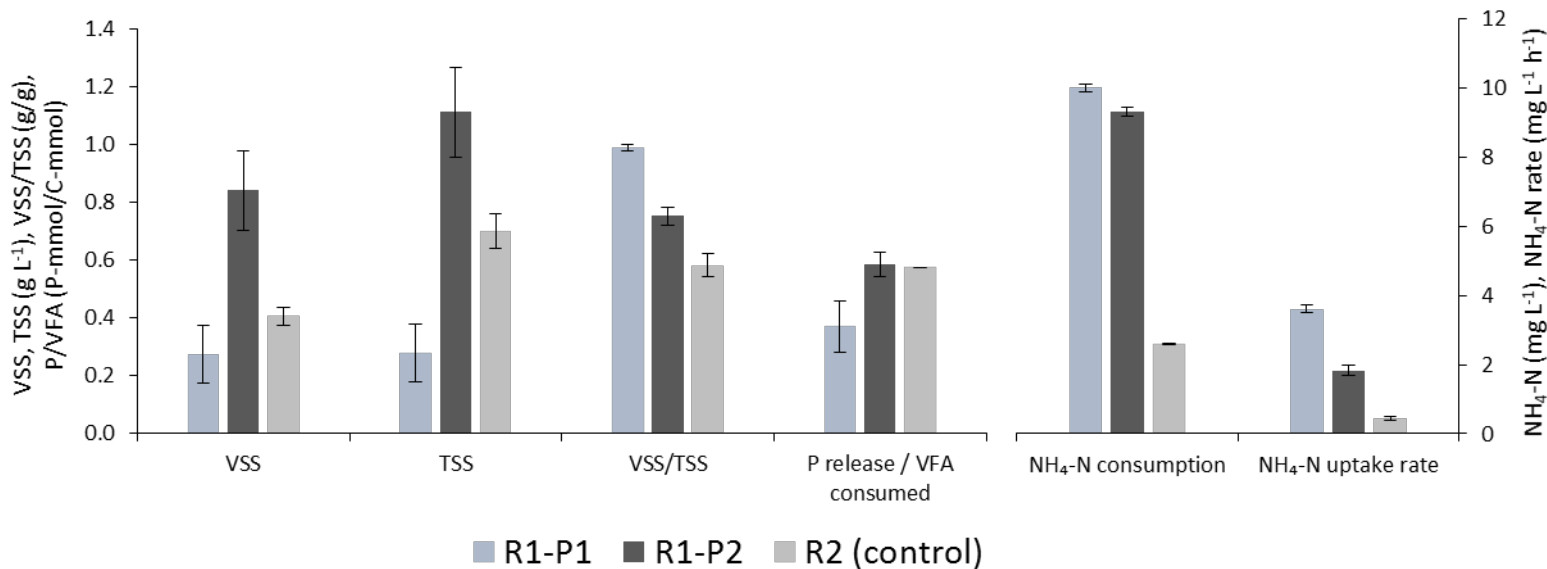
373
 374 In P2, there was a reduction in the DO concentration in the middle of the aerobic stage, which
 375 coincided with the depletion of inorganic carbon (Fig. 6) as microalgae were no longer able to
 376 perform photosynthesis in the absence of inorganic carbon. This indicates that limiting the
 377 microalgae growth by controlling the inorganic carbon was a successful measure. The medium
 378 (COD: HCO_3^- : $\text{NH}_4\text{-N}$) in P2 was supplied at a ratio of 10:10:4, but the actual consumption ratio
 379 was 10:10:1, which was similar to that predicted by the model when considering that PAOs

380 contributed part of HCO_3 as CO_2 (ratio of 100:88:10; Fig. 1-a,b). PAOs managed to contribute
381 $8.03 \text{ mg CO}_2 \text{ L}^{-1}$ (11.13 mg L^{-1} as HCO_3) anaerobically (Fig. 6), which was almost similar to the
382 model estimation of $11 \text{ mg CO}_2 \text{ L}^{-1}$ (Table A3, Appendix A). The initial approach adopted in P1
383 was to avoid limiting conditions for microalgae growth, and therefore inorganic carbon was
384 supplied in abundance in P1 (not considering the share provided by PAOs). This measure
385 potentially generated excess microalgal biomass in P1 which outcompeted PAOs for $\text{NH}_4\text{-N}$.

386 **3.3 EBPR-PAS system performance and characteristics**

387 At steady-state, an enmeshed mixed culture of PAOs and microalgae was obtained from R1 in P2
388 (Appendix C). The average TSS concentration was $1108.06 \pm 154.9 \text{ mg L}^{-1}$ (SE= 41.4, n=17), with
389 an average VSS of $839.18 \pm 137 \text{ mg L}^{-1}$ (SE= 33.24, n= 17) and a VSS/TSS ratio of 0.75 ± 0.027
390 (SE= 0.007, n=17; Fig. 7), which was close to the model predictions (Fig. 1-c). The lower
391 VSS/TSS ratio in P2 (0.75) than in P1 (0.99; Fig. 7) was an indicator of improving EBPR activities
392 in R1 during P2. PAOs generate inactive biomass/inert suspended solids (ISS) from the stored
393 poly-P and associated counter ions (Mg^{+2} , K^{+1} , and Ca^{+2}), and therefore have a low VSS/TSS ratio
394 (Ekama and Wentzel, 2004), while microalgae generate less ISS. Consequently, the VSS/TSS ratio
395 is lower for a conventional EBPR system (R2) than an EBPR-PAS system (R1). This was apparent
396 in the current study and that of Carvalho et al. (2018), as respective VSS/TSS ratios of 0.75 (Fig.
397 7) and 0.68-0.8 were recorded for EBPR-PAS systems. A VSS/TSS ratio of 0.58 was achieved in
398 R2 of the current study (Fig. 7) and by Welles et al. (2015) for a conventional EBPR system.

399 Phosphorus release/VFA consumed was $0.58 \pm 0.04 \text{ P-mmol/C-mmol}$ during P2, which was
400 similar to R2 (Fig. 7). This was also an indication of good EBPR activity according to the results
401 of Welles et al. (2017) and Saad et al. (2016), who reported values of between 0.4 and 0.8 P-
402 mmol/C-mmol for PAOs in conventional EBPR systems.



403 **Fig. 7** VSS, TSS, VSS/TSS, P-release/VFA consumed, total NH₄-N consumption, and NH₄-N
 404 uptake rate for the EBPR-PAS system (R1) during P1 and P2, and the control reactor (conventional
 405 EBPR system, R2)

406

407 During P2, COD consumed/average P net removal for R1 (100/10.4) was lower than R2 (100/8).

408 This indicates that the EBPR-PAS system required less organic carbon than the conventional

409 EBPR system. On days 62, 63, 64, 68, 69, and 76, the system was capable of complete PO₄

410 removal, without the supply of any external aeration (Appendix D, Fig. D.1, Fig. D.2), and with a

411 low COD/P net removal ratio of 100/12 in which PAOs and microalgae account for P removal of

412 11 and 1 mg L⁻¹, respectively, according to the model (Fig. 1-a). The COD/P net removal of 100/12

413 was lower than that achieved in the literature for best performing conventional EBPR systems. For

414 example, Carvalheira et al. (2014a) achieved a COD consumed/P net removal of 200/20, with an

415 influent P concentration of 20 mg L⁻¹ for conventional EBPR reactor. Carvalho et al. (2018) also

416 reported very low COD consumed/P net removal (200/34) for an EBPR-PAS system, with an

417 influent P concentration of 60 mg L⁻¹. The main reason for this low COD/P net removal for an

418 EBPR-PAS system was that microalgae participated in P removal (1.3 % of microalgae biomass),

419 without requiring COD (with inorganic carbon as the carbon source). In contrast, the EBPR-PAS
420 system of the current study (R1) and Carvalho et al. (2018) had a lower capacity to remove
421 phosphate per g biomass (P/VSS) than the conventional EBPR system (R2). This because PAOs
422 can uptake P to a maximum of 38% of their biomass (Wentzel et al., 1990), while microalgae
423 typically can only uptake P to 1.3% of their biomass (Mara, 2004). However, there are some types
424 of microalgae/cyanobacteria capable of acting as PAOs that can significantly contribute to poly-P
425 storage. For example, Ji et al. (2020b) found that the cyanobacteria *Pantanalinema spp.* were the
426 major phosphorus-accumulating organisms in microalgal-bacterial granular sludge, although the
427 P content of this cyanobacteria was less than 38% of VSS like PAOs. Therefore, for similar
428 amounts of phosphate removal, an EBPR-PAS system requires less organic carbon and generates
429 higher biomass than a conventional EBPR system.

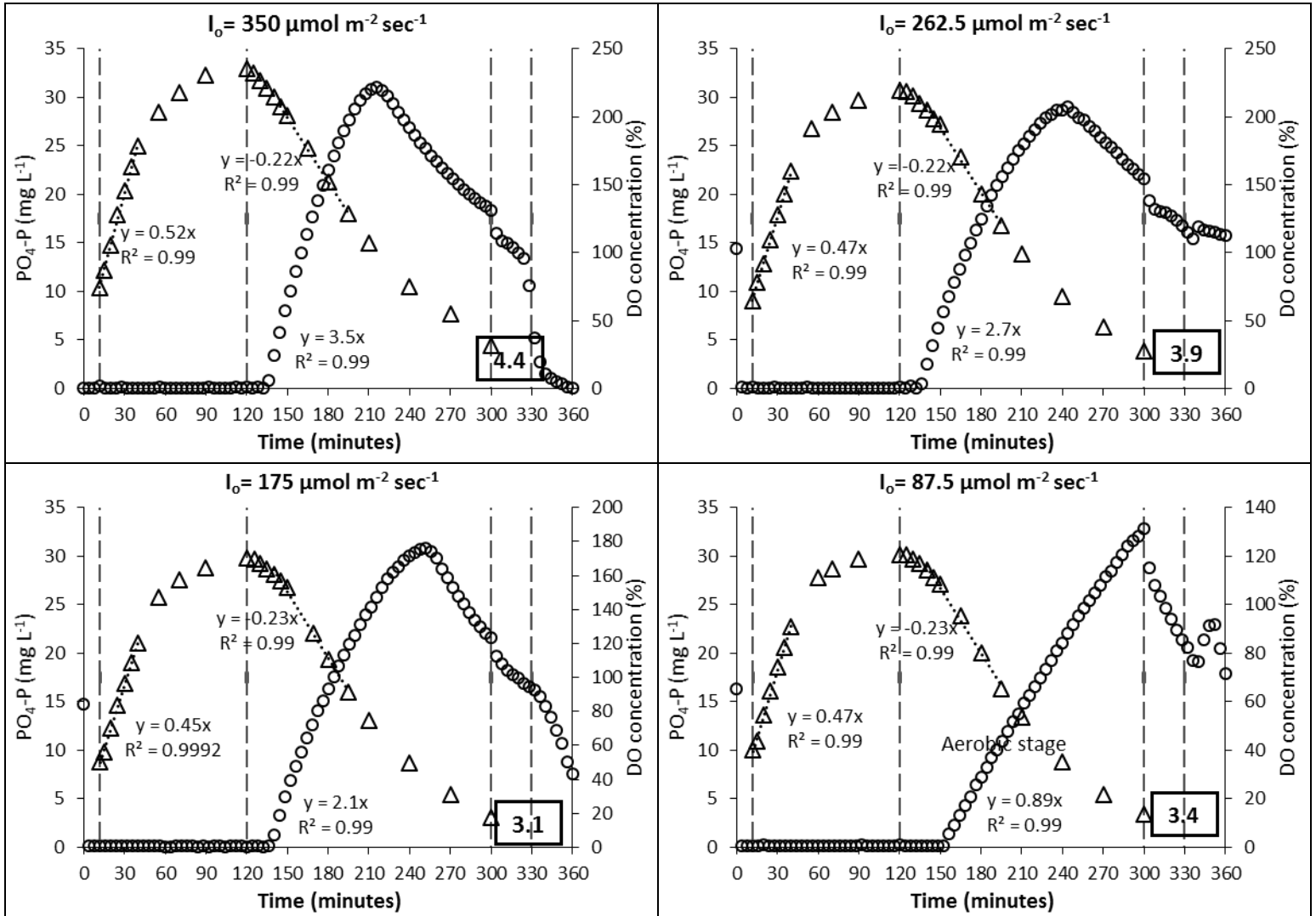
430 The total $\text{NH}_4\text{-N}$ consumption by biomass (microalgae + PAOs) was $9.3 \pm 0.74 \text{ mg L}^{-1}$ (SE= 0.25,
431 $n= 11$) during P2 (Fig. 7). This value was approximately equal to that predicted by the model (10
432 mg L^{-1}), in which PAOs and microalgae account for $\text{NH}_4\text{-N}$ uptake of 3.04 and 6.96 mg L^{-1} ,
433 respectively (Fig. 1-a).

434

435 **3.4 Third experimental phase (light assessment and optimization)**

436 The reactor performance for different light intensities is shown in Fig. 8. Three of the four light
437 intensities tested were higher than the model estimated light requirement for photosynthesis (145
438 $\mu\text{mol m}^{-2} \text{ sec}^{-1}$). The oxygen production rate by microalgae decreased when the light intensity
439 reduced (3.5, 2.7, 2.1, and 0.89 % saturated DO min^{-1} for light intensities of 350, 262.5, 175, and
440 $87.5 \mu\text{mol m}^{-2} \text{ sec}^{-1}$, respectively; linear correlation: $R= 0.99$; Fig. 8). Yet, the overall performance
441 between light intensities was not significant ($p > 0.05$). No significant variation was observed in

442 the reactor performance regarding P release and P uptake (Fig. 8), suggesting that the culture
 443 selected in P2 conditions was resilient to inhibition by lower light intensities.



444 **Fig. 8** $PO_4\text{-P}$ profiles (Δ) combined with online DO profiles (\circ) for incident light intensities:
 445 350, 262.5, 175, and 87.5 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ for R1 on days: 83, 86, 89 and 92 of operation,
 446 respectively.

447

448 Reducing the light in P1 (e.g. from 350 to 87.5 $\mu\text{mol m}^{-2} \text{sec}^{-1}$) may limit the microalgae growth
 449 without the need to reduce the inorganic carbon as implemented in P2. This measure would
 450 possibly reduce the uptake rate of $NH_4\text{-N}$ by microalgae (and therefore delay the early
 451 consumption of $NH_4\text{-N}$ that occurred in P1). As the light intensity was decreased, the P

452 concentration in the effluent improved slightly (Fig. 8). Carvalheira et al. (2014b) observed that
453 PAOs in an EBPR system prefer to grow in low DO concentration, and outnumber GAOs.
454 Therefore, too much light may not be beneficial for PAOs. Conversely, too little light may also
455 not be favourable for PAOs, as the total oxygen produced by microalgae may not meet the
456 metabolic and anabolic requirement of PAOs as explained by the model of Smolders et al. (1994b).

457

458 **3.5 Limitations and implications of the study**

459 An EBPR-PAS system exploits the synergistic relationship between microalgae and PAOs.
460 Uncoupling HRT from SRT facilitates the use of a smaller sized system (HRT = 12 h) than other
461 microalgal-bacterial systems (commonly 2-6 days for municipal wastewater; Muñoz and
462 Guieysse, 2006; Anbalagan et al. 2016). Also, the EBPR-PAS system requires no external aeration,
463 has a high capacity to remove P at low influent COD concentrations, and significantly reduces
464 CO₂ footprint. However, the current study showed that the system is not always successful and its
465 performance depends on the composition of the influent wastewater. This may have practical
466 implications that are difficult to discern at full-scale with real wastewater applications. For
467 example, municipal wastewater will demonstrate an intrinsic temporal variability (Metcalf and
468 Eddy, 2003), while the system described in our study treated wastewater at a steady
469 COD:HCO₃:NH₄-N ratio of 10:10:4.

470 The current study was operated without nitrification. Introducing nitrifiers in the EBPR-PAS
471 system has different implications. The system will require more O₂ as nitrification usually
472 demands intensive aeration (4.57 mg O₂ mg⁻¹ NH₄-N nitrified; Wiesmann, 1994). In addition, the
473 nitrification process consumes a considerable amount of alkalinity (8.71 mg HCO₃ mg⁻¹ NH₄-N
474 nitrified; Wiesmann, 1994), therefore, the system will require more HCO₃ to produce more O₂ and

475 to meet this demand. However, denitrification can recover half the O_2 and alkalinity lost in
476 nitrification ($2.86 \text{ mg } O_2 \text{ mg}^{-1} \text{ NO}_3\text{-N}$ de-nitrified; $4.36 \text{ mg HCO}_3 \text{ mg}^{-1} \text{ NO}_3\text{-N}$ de-nitrified;
477 Wiesmann, 1994). Some PAOs are assumed to use nitrate as an external electron acceptor,
478 allowing efficient integration of simultaneous nitrogen and phosphate removal with minimal
479 organic carbon (COD) requirements (Sorm et al., 1996; Saad et al., 2016). Overall, less organic
480 carbon, more nitrogen, and more inorganic carbon are expected for PAOs-microalgae-nitrifier
481 symbiosis than PAOs-microalgae symbiosis.

482 The EBPR-PAS system in this study favored P removal even at low light intensities, which is an
483 indication that the system can be operated at low power consumption if artificial lighting is used.
484 However, when nitrification is incorporated in the EBPR-PAS system, more light is expected to
485 be required by microalgae to meet the high DO demand by nitrifiers. In addition, further light is
486 required as a result of increased TSS concentration caused by the additional biomass of nitrifiers,
487 although the additional biomass will be minimal as nitrifiers have a very small yield (0.1 mg VSS
488 $\text{mg}^{-1} \text{ NH}_4\text{-N}$; Ekama and Wentzel, 2008). The light path and culture density (as controlled by SRT)
489 are potential controls to manipulate the photo-oxygenation rate (Arashiro et al., 2017; Rada-Ariza
490 et al., 2019). For example, decreasing the light path and reducing SRT can maximize the DO
491 concentration in the system.

492

493 **4. Conclusion**

494 This study showed that careful control of $\text{NH}_4\text{-N}$ and HCO_3 is critical to balancing PAOs and
495 microalgae populations in the EBPR-PAS system. At a COD: HCO_3 : $\text{NH}_4\text{-N}$ ratio of 10:20:1, the
496 EBPR-PAS system favored nitrogen removal and microalgal growth, and exhibited poor EBPR
497 activities. At this ratio, the growth of PAOs was likely inhibited because microalgae consume

498 ammonium earlier than PAOs during the illuminated stage of operation. However, once the
499 COD:HCO₃:NH₄-N ratio was changed to 10:10:4, the PAOs and microalgae populations were
500 balanced, and the system performance improved significantly to remove P. The study also revealed
501 that there were no significant differences in the system performance for different light intensities,
502 suggesting that the mixed culture was robust against light fluctuations. Future studies should focus
503 on testing the EBPR-PAS system on real municipal wastewater in large volumes to demonstrate
504 applicability of this system to full scale operations. In addition, the incorporation of nitrification
505 and the influence of nitrate on PAOs-microalgae interplay should be investigated further. Finally,
506 the long-term effect of light intensity should be studied to investigate the system performance for
507 stable reactor operation.

508

509

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514

515 **References**

516 Abouhend AS, McNair A, Kuo-Dahab WC, Watt C, Butler CS, Milferstedt K, Hamelin Jrm, Seo
517 J, Gikonyo GJ, El-Moselhy KM (2018) The oxygenic photogranule process for aeration-
518 free wastewater treatment. *Environmental science & technology* 52: 3503-3511

519

520 Ahmad JSM, Cai W, Zhao Z, Zhang Z, Shimizu K, Lei Z, Lee D-J (2017) Stability of algal-
521 bacterial granules in continuous-flow reactors to treat varying strength domestic
522 wastewater. *Bioresource technology* 244: 225-233

523

524 Almada-Calvo F (2014) Effect of temperature, dissolved inorganic carbon and light intensity on
525 the growth rates of two microalgae species in monocultures and cocultures. PhD thesis,
526 University of Texas at Austin.
527

528 Anbalagan A, Schwede S, Lindberg C-F, Nehrenheim E (2016) Influence of hydraulic retention
529 time on indigenous microalgae and activated sludge process. *Water research* 91: 277-284
530
531

532 Anbalagan A, Toledo-Cervantes A, Posadas E, Rojo EM, Lebrero R, González-Sánchez A,
533 Nehrenheim E, Muñoz R (2017) Continuous photosynthetic abatement of CO₂ and volatile
534 organic compounds from exhaust gas coupled to wastewater treatment: evaluation of
535 tubular algal-bacterial photobioreactor. *Journal of CO₂ Utilization* 21: 353-359
536

537 APHA (2005) Standard methods for the examination of water and wastewater. American Public
538 Health Association (APHA): Washington, DC, USA
539

540 Arashiro LT, Rada-Ariza AM, Wang M, Van Der Steen P, Ergas SJ (2017) Modelling shortcut
541 nitrogen removal from wastewater using an algal–bacterial consortium. *Water Science and
542 Technology* 75: 782-792
543

544 Bashar R, Gungor K, Karthikeyan K, Barak P (2018) Cost effectiveness of phosphorus removal
545 processes in municipal wastewater treatment. *Chemosphere* 197: 280-290
546

547 Becker EW (1994) *Microalgae: biotechnology and microbiology* Cambridge University Press
548

549 Belkin S, Boussiba S (1991) Resistance of *Spirulina platensis* to ammonia at high pH values. *Plant
550 and cell physiology* 32: 953-958
551

552 Brdjanovic D, van Loosdrecht MC, Hooijmans CM, Alaerts GJ, Heijnen JJ (1998) Minimal
553 aerobic sludge retention time in biological phosphorus removal systems. *Biotechnology
554 and bioengineering* 60: 326-332
555
556

557 Carvalheira M, Oehmen A, Carvalho G, Reis MA (2014a) Survival strategies of polyphosphate
558 accumulating organisms and glycogen accumulating organisms under conditions of low
559 organic loading. *Bioresource technology* 172: 290-296
560

561 Carvalheira M, Oehmen A, Carvalho G, Eusébio M, Reis MA (2014b) The impact of aeration on
562 the competition between polyphosphate accumulating organisms and glycogen
563 accumulating organisms. *Water research* 66: 296-307
564

565 Carvalho V, Freitas E, Silva P, Fradinho J, Reis M, Oehmen A (2018) The impact of operational
566 strategies on the performance of a photo-EBPR system. *Water research* 129: 190-198
567

568 Chudoba J, Ottova V, Madera V (1973) Control of activated sludge filamentous bulking—I. Effect
569 of the hydraulic regime or degree of mixing in an aeration tank. *Water Research* 7: 1163-
570 1182
571

572 Comeau Y, Hall K, Hancock R, Oldham W (1986) Biochemical model for enhanced biological
573 phosphorus removal. *Water Research* 20: 1511-1521
574

575 Craggs R, Davies-Colley R, Tanner C, Sukias J (2003) Advanced pond system: performance with
576 high rate ponds of different depths and areas. *Water Science and Technology* 48: 259-267
577

578 Craggs R, Park J, Heubeck S, Sutherland D (2014) High rate algal pond systems for low-energy
579 wastewater treatment, nutrient recovery and energy production. *New Zealand Journal of*
580 *Botany* 52: 60-73
581

582 Ekama G, Wentzel M (2004) A predictive model for the reactor inorganic suspended solids
583 concentration in activated sludge systems. *Water Research* 38: 4093-4106
584

585 Ekama GA, Wentzel MC (2008) Nitrogen removal. *Biological wastewater treatment IWA*
586 *Publishing, London, UK, pp. 87-138.*
587

588 EUMETSAT (2020) https://www.eumetsat.int/website.home/News/DAT_2187685.html (May,
589 2020)
590

591 Henze M, Comeau Y (2008) Wastewater characterization. *Biological wastewater treatment:*
592 *Principles modelling and design: 33-52*
593

594 Henze M, van Loosdrecht MC, Ekama GA, Brdjanovic D (2008) *Biological wastewater treatment*
595 *IWA publishing*
596

597 Ji B, Zhang M, Gu J, Ma Y, Liu Y (2020a) A self-sustaining synergetic microalgal-bacterial
598 granular sludge process towards energy-efficient and environmentally sustainable
599 municipal wastewater treatment. *Water Research: 115884*
600

601 Ji B, Zhang M, Wang L, Wang S, Liu Y (2020b) Removal mechanisms of phosphorus in non-
602 aerated microalgal-bacterial granular sludge process. *Bioresource Technology: 123531*
603

604 Karya N, Van der Steen N, Lens P (2013) Photo-oxygenation to support nitrification in an algal-
605 bacterial consortium treating artificial wastewater. *Bioresource technology* 134: 244-250
606

607 Lopez-Vazquez CM, Oehmen A, Hooijmans CM, Brdjanovic D, Gijzen HJ, Yuan Z, van
608 Loosdrecht MC (2009) Modeling the PAO–GAO competition: effects of carbon source,
609 pH and temperature. *Water Research* 43: 450-462
610

611

612 Manser ND, Wang M, Ergas SJ, Mihelcic JR, Mulder A, van de Vossenberg J, van Lier JB, van
613 der Steen P (2016) Biological Nitrogen Removal in a Photosequencing Batch Reactor with

614 an Algal-Nitrifying Bacterial Consortium and Anammox Granules. *Environmental Science*
615 & *Technology Letters* 3: 175-179
616

617 Mara D (2004) *Domestic Wastewater Treatment in Developing Countries* Earthscan
618

619 Martins AM, Heijnen JJ, van Loosdrecht MC (2004) Bulking sludge in biological nutrient removal
620 systems. *Biotechnology and bioengineering* 86: 125-135
621

622 Metcalf and Eddy, Inc. (2003) *Wastewater engineering: treatment and reuse*. 4th ed., McGraw-
623 Hill, New York.
624

625 Munoz R, Guieysse B (2006) algal-bacterial processes for the treatment of hazardous
626 contaminants: a review. *Water research* 40: 2799-2815
627

628 NEN 6472 (1983) *Water-Photometric determination of ammonium content Dutch Normalization*
629 Institute Delft.
630

631 NEN 6520 (1982). *Water: Spectrophotometric Determination of Chlorophyll-a Content*
632 *Nederlands Normalisatie-instituut, Delft, the Netherlands*
633

634 Nielsen P, Daims H (2009) *FISH handbook for biological wastewater treatment* Iwa publishing
635
636

637 Rada-Ariza A, Fredy D, Lopez-Vazquez C, Van der Steen N, Lens P (2019) Ammonium removal
638 mechanisms in a microalgal-bacterial sequencing-batch photobioreactor at different solids
639 retention times. *Algal Research* 39: 101468
640

641 Rada-Ariza A, Lopez-Vazquez C, Van der Steen N, Lens P (2017) Nitrification by microalgal-
642 bacterial consortia for ammonium removal in flat panel sequencing batch photo-
643 bioreactors. *Bioresource technology* 245: 81-89
644

645 Rosso D, Larson LE, Stenstrom MK (2008) Aeration of large-scale municipal wastewater
646 treatment plants: state of the art. *Water Science and Technology* 57: 973-978
647

648 Saad SA, Welles L, Abbas B, Lopez-Vazquez CM, van Loosdrecht MC, Brdjanovic D (2016)
649 Denitrification of nitrate and nitrite by ‘*Candidatus Accumulibacter phosphatis*’ clade IC.
650 *Water Research* 105: 97-109
651

652 Schuler A, Jenkins D, Ronen P (2001) Microbial storage products, biomass density, and settling
653 properties of enhanced biological phosphorus removal activated sludge. *Water science and*
654 *technology* 43: 173-180
655

656 Smolders G, Van der Meij J, Van Loosdrecht M, Heijnen J (1994a) Model of the anaerobic
657 metabolism of the biological phosphorus removal process: stoichiometry and pH influence.
658 *Biotechnology and bioengineering* 43: 461-470
659

660 Smolders G, Van der Meij J, Van Loosdrecht M, Heijnen J (1994b) Stoichiometric model of the
661 aerobic metabolism of the biological phosphorus removal process. *Biotechnology and*
662 *bioengineering* 44: 837-848
663

664 Sorm R, Bortone G, Saltarelli R, Jenicek P, Wanner J, Tilche A (1996) Phosphate uptake under
665 anoxic conditions and fixed-film nitrification in nutrient removal activated sludge system.
666 *Water Research* 30: 1573-1584
667

668 Sutherland DL, Turnbull MH, Broady PA, Craggs RJ (2014) Effects of two different nutrient loads
669 on microalgal production, nutrient removal and photosynthetic efficiency in pilot-scale
670 wastewater high rate algal ponds. *Water research* 66: 53-62
671

672 Swinehart DF (1962) The beer-lambert law. *Journal of chemical education* 39: 333
673

674 van der Steen P, Rahsilawati K, Rada-Ariza AM, Lopez-Vazquez CM, Lens PN (2015) A new
675 photo-activated sludge system for nitrification by an algal-bacterial consortium in a photo-
676 bioreactor with biomass recycle. *Water Science and Technology* 72: 443-450
677

678 van Loosdrecht MC, Nielsen PH, Lopez-Vazquez CM, Brdjanovic D (2016) *Experimental*
679 *methods in wastewater treatment* IWA publishing
680

681 Welles L, Abbas B, Sorokin D, Lopez-Vazquez C, Hooijmans C, van Loosdrecht M, Brdjanovic
682 D (2017) Metabolic Response of “*Candidatus Accumulibacter Phosphatis*” Clade II C to
683 Changes in Influent P/C Ratio. *Front Microbiol* 7: 2121 doi: 103389/fmicb
684

685 Welles L, Tian W, Saad S, Abbas B, Lopez-Vazquez C, Hooijmans C, van Loosdrecht M,
686 Brdjanovic D (2015) *Accumulibacter* clades Type I and II performing kinetically different
687 glycogen-accumulating organisms metabolisms for anaerobic substrate uptake. *Water*
688 *research* 83: 354-366
689

690 Wentzel MC, Comeau Y, Ekama GA, van Loosdrecht MC, Brdjanovic D (2008) Enhanced
691 biological phosphorus removal. *Biological Wastewater Treatment-Principles, Modelling*
692 *and Design*, ed Mogens Henze: 155-220
693

694 Wentzel M, Ekama G, Dold P, Marais G (1990) Biological excess phosphorus removal-steady
695 state process design. *Water Sa* 16: 29-48
696

697

698 Wiesmann U (1994) Biological nitrogen removal from wastewater *Biotechnics/wastewater*:113-
699 154.
700

701 Wilderer PA, Irvine RL, Goronszy MC (2001) *Sequencing batch reactor technology* IWA
702 publishing
703

704 Wolf G, Picioreanu C, van Loosdrecht M (2007) Kinetic modeling of phototrophic biofilms: the
705 PHOBIA model. *Biotechnology and bioengineering* 97: 1064-1079

