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Published in:
Algal Research

Link to article, DOI:
[10.1016/j.algal.2016.10.010](https://doi.org/10.1016/j.algal.2016.10.010)

Publication date:
2016

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Wágner, D. S., Radovici, M., Smets, B. F., Angelidaki, I., Valverde Pérez, B., & Plósz, B. G. (2016). Harvesting microalgae using activated sludge can decrease polymer dosing and enhance methane production via co-digestion in a bacterial-microalgal process. *Algal Research*, 20, 197-204. DOI: 10.1016/j.algal.2016.10.010

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1 Harvesting microalgae using activated sludge can decrease polymer dosing and
2 enhance methane production via co-digestion in a bacterial-microalgal process

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7 **Abstract**

8 Third generation biofuels, e.g. biofuels production from algal biomass, have gained attention due to
9 increased interest on global renewable energy. However, crop-based biofuels compete with food
10 production and should be avoided. Microalgal cultivation for biofuel production offers an
11 alternative to crops and can become economically viable when combined with the use of used water
12 resources. Besides nutrients and water, harvesting microalgal biomass represents one of the major
13 costs related to biofuel production and thus efficient and cheap solutions are needed. In bacterial-
14 algal systems, there is the potential to produce energy by co-digesting the two biomasses. We
15 present an innovative approach to recover microalgal biomass via a two-step flocculation using
16 bacterial biomass after the destabilization of microalgae with conventional cationic polymer. A
17 short solids retention time (SRT) enhanced biological phosphorus removal (EBPR) system was
18 combined with microalgal cultivation. Two different bacterial biomass removal strategies were
19 assessed whereby bacterial biomass was collected from the solid-liquid separation after the
20 anaerobic phase and after the aerobic phase. Microalgal recovery was tested by jar tests where three
21 different chemical coagulants in coagulation-flocculation tests (AlCl_3 , PDADMAC and Greenfloc
22 120) were assessed. Furthermore, jar tests were conducted to assess the microalgal biomass
23 recovery by a two-step flocculation method, involving chemical coagulants in the first step and

24 bacterial biomass used in the second step to enhance the flocculation. Up to 97 % of the microalgal
25 biomass was recovered using 16 mg polymer/g algae and 0.1 g algae/g bacterial biomass.
26 Moreover, the energy recovery by the short-SRT EBPR system combined with microalgal
27 cultivation was assessed via biomethane potential tests. Up to 560±24 ml CH₄/gVS methane yield
28 was obtained by co-digesting bacterial biomass collected after the anaerobic phase and microalgal
29 biomass. The energy recovery obtained from the short-SRT EBPR system is about 40% of the
30 influent chemical energy.

31 **Keywords**

32 Green microalgae; enhanced biological phosphorus removal; bioflocculation; co-digestion; energy
33 recovery

34 **1. Introduction**

35 Due to the challenges related to greenhouse gas emissions, decreasing fossil fuel reserves and
36 global and national pressure, new solutions are sought to produce renewable energy including the
37 use of biomass for biofuel production. However, first generation biofuels (derived from agricultural
38 crops) are of questionable sustainability as they compete for land with food crops, thereby affecting
39 the global food security [1,2]. Similarly, second generation biofuels, e.g. non-food energy crops
40 (e.g. vegetative grasses or short rotation forests), agricultural and forest residues, compete for land
41 use in some cases and there are technological difficulties related to the conversion processes [1].
42 Third generation biofuels such as microalgae have the advantages that they can be produced all year
43 round, do not compete food production as they can be grown on non-arable land, have rapid growth
44 rates and the biochemical composition can be manipulated by varying cultivation conditions and
45 strains [1,3]. The cultivation of microalgae for biofuel production can be economically viable when

46 coupled with wastewater treatment [3–6] which provides the water and nutrients (nitrogen and
47 phosphorous) required for growth [7].

48 Conventional wastewater treatment has a high energy demand required mainly by the aeration
49 process whereby organic carbon present in wastewater is oxidized to CO₂ and nitrification takes
50 place under long sludge ages [8]. This leads to the loss of the energy potential of the activated
51 sludge [9] together with the loss of nutrients (nitrogen and phosphorus) [8]. Short solids retention
52 time (SRT) activated sludge systems propose a solution whereby rather than the oxidization of
53 organic carbon, activated sludge preserves the organic carbon promoting higher potential for energy
54 recovery [10].

55 Bacterial-algal systems can be coupled with wastewater treatment, whereby nutrients and energy
56 can be recovered [3]. In a novel wastewater resource recovery approach, Valverde-Pérez et al. [11]
57 proposed an enhanced biological phosphorus recovery and removal (EBP2R) process, able to
58 provide optimal culture media for downstream microalgal cultivation. The system referred to as
59 TRENS, consists of a modified short-SRT EBP2R process where an additional solid-liquid
60 separation is included after the anaerobic phase (Fig. S1, Supporting Information, SI). Under
61 anaerobic conditions, phosphorus accumulating organisms (PAO) take up the volatile fatty acids
62 (VFA) from the wastewater and store them as polyhydroxyalkanoates (PHA) intracellularly while
63 releasing intracellular phosphorus (poly-P) [12]. Under aerobic conditions the stored PHA are used
64 to produce energy for biomass growth as well as phosphorus uptake and storage [12]. Thus, the
65 effluent water after the solid-liquid separation after the anaerobic phase is rich in phosphorus, whilst
66 the effluent after the solid-liquid separation after the aerobic phase is rich in nitrogen. The short-
67 SRT EBP2R can provide optimal cultivation medium to a downstream photobioreactor (PBR) by
68 mixing the phosphorus and nitrogen rich effluent streams in an optimal ratio.

69 When microalgal cultivation is coupled with wastewater treatment the lipid content of the
70 microalgae is fairly low (4.9-11.3%) due to the relatively high nutrients supplied [3,13]. It is
71 energetically favourable to apply anaerobic digestion when the lipid concentration is lower than
72 40% [14]. In addition, anaerobic digestion is applicable for biomasses with high moisture content
73 (80-90%), which makes it suitable for microalgal biomass conversion [1,15]. Thus, anaerobic
74 digestion is the preferred route over biodiesel production when energy recovery is considered from
75 microalgae cultivated on wastewater resources [13]. The nutrient rich effluents of the anaerobic
76 digestion can be used for further cultivation of microalgae [1]. Anaerobic digesters are in many
77 cases available in the existing wastewater treatment plants and biogas production can be increased
78 by co-digestion of microalgae and activated sludge [16]. Nonetheless, not all microalgal species are
79 suitable for biogas production, mainly due to their cell wall structure and their high nitrogen content
80 [14,17].

81 A C/N ratio of 20 (g/g) is suitable for optimal digestion conditions [4,18]. While, in freshwater
82 microalgae it is typically around 10 [14,19]. Many studies proposed co-digestion with other
83 biomass sources, e.g. activated sludge, to improve digestibility by balancing the C/N ratio, thereby
84 providing optimal nutrient balance for enhanced methane yield [3,15,16,18]. Additionally, the co-
85 digestion of various waste lines reduce costs by using a single anaerobic digester unit for digestion
86 of multiple substrates [3].

87 The major bottleneck of microalgal cultivation for biogas production is the cost related to biomass
88 harvesting [15,20,21]. Energy-intensive and expensive methods, e.g. centrifugation or membrane
89 technologies [20], are only applicable when the biomass is used to produce high value products
90 [21]. Thus simple harvesting methods are required for reliable and safe downstream applications
91 [3].

92 Flocculation is an alternative and cheap harvesting method [20,22]. During coagulation the negative
93 surface charge of microalgae, caused largely by the presence of carboxyl groups, is destabilized.
94 This is followed by a second flocculation step whereby aggregates are formed, thus promoting more
95 effective gravity sedimentation [21,23]. Iron or aluminium salts, which form positively charged
96 hydroxides when dissolved in water, are successfully used as coagulants that neutralize the negative
97 algal cells promoting aggregate formation [24]. $AlCl_3$ addition is a common method in wastewater
98 treatment to enhance the coagulation-flocculation process [25]. Nevertheless, aluminium salts
99 require high dosage and the downstream usage is limited due to toxicity [21]. Cationic polymers can
100 induce flocculation of algal biomass by surface charge neutralization or by inter-cellular bridging
101 [24]. The effectiveness of the polymers depends on their size and charge density. Compared to
102 metal salts, polymers usually operate at lower dosages [21]. Flocculation efficiency by polymers
103 declines at high dosages due to restabilisation [20,21]. Bioflocculation has also been proposed: in
104 this case a specific bacteria, fungi or algae are added to the microalgal culture promoting
105 flocculation [20,26].

106 Bacterial-algal systems have the potential to recover energy through biomass production. Thus, a
107 cost-effective harvesting method is needed whereby the algal and bacterial biomass can be
108 recovered. The objectives of this study are (i) to test the effect of different chemical flocculants on
109 microalgal recovery; (ii) to develop a cost-effective method of harvesting microalgae via a two-step
110 flocculation using cationic polymer for destabilisation of microalgae and bacterial biomass from a
111 short-SRT EBPR system to enhance the aggregation of the algae; (iii) to optimize the cationic
112 polymer dosing; (iv) to assess the effect of different algae/bacterial biomass ratios and the effect of
113 bacterial biomass settleability on algal biomass recovery; and (v) to assess the methane production
114 potential by co-digestion of the harvested bacterial-algal biomass.

115 **2. Materials and methods**

116 **2.1. Microalgal cultivation and EBPR operation**

117 *2.1.1. Algal biomass used for pre-testing different coagulants*

118 We cultivated a mixed green microalgal consortium consisting mainly of *Chlorella sorokiniana* and
119 *Scenedesmus sp.* (see Wágner et al. [27]). The consortium was cultivated with effluent water from
120 the Lundtofte WWTP (Denmark). Ammonium and phosphorus were spiked to reach 20 mg/L NH₄-
121 N and 2.75 mg/L PO₄-P (16 N-to-P ratio) in the microalgal batch cultivation. 2 L glass reactors
122 were used to cultivate the algae with constant stirring at 180 rpm using magnetic stirrers and with
123 aeration with CO₂ enriched air (5 % CO₂) at a flow rate of 10 L/h. Light was supplied from the two
124 sides of the batches with fluorescent lamps (18 W, GroLux, Sylvania®, USA), providing 160 μmol
125 photons m⁻² s⁻¹ continuously. The temperature in the room was regulated at 20 °C. 80% of the algal
126 suspension was removed every 2-3 days from the batch reactor and the reactor was refilled with
127 new effluent water. The pH of the algal culture varied between 6.84 and 7.95 during the
128 experiments. The TSS of the algal suspension used for flocculation varied between 0.29 and 0.37
129 g/L. The algal TSS and OD values used for each flocculation experiment are reported in Table S1,
130 SI.

131 *2.1.2. Algal and bacterial biomass used for the two-step flocculation*

132 The same mixed green microalgal consortium was used in the two-step flocculation experiments.
133 The microalgal culture was grown on effluent water from a laboratory scale EBPR system [28]
134 operated at 3-3.5 days SRT as a sequencing batch reactor (SBR) (fed with pre-clarified wastewater
135 from Lundtofte WWTP, Denmark). The ammonium and ortho-phosphate concentrations were
136 adjusted to an N/P molar ratio of 17 in the beginning of each microalgal batch (adjusted to 23 mg/L

137 NH₄-N and 3 mg/L PO₄-P). 1.5 L glass reactors were used to cultivate the algae with constant
138 aeration with CO₂ enriched air (5 % CO₂) at a flow rate of 10 L/h. Light was supplied from the top
139 of the batch reactor continuously with a custom-built lamp, providing 500 μmol photons m⁻² s⁻¹,
140 with a metal-halide light bulb (OSRAM©, Germany). The reactors were kept at room temperature.
141 The pH of the algal culture varied in the range of 7 – 8.5 during the experiments. 60% of the algal
142 suspension was removed every 2-3 days and the batch reactor was refilled with new effluent water
143 from the EBPR system (adjusted to N/P molar ratio of 17). The TSS of the algal suspension varied
144 in the range of 0.27 - 0.52 g/L during the experiments. The algal TSS and OD values used for each
145 flocculation experiment are reported in Table S1, SI. The bacterial biomass was taken from the
146 short-SRT EBPR system using two biomass removal strategies: i) bacterial biomass removed at the
147 end of the anaerobic phase; ii) bacterial biomass removed at the end of the aerobic phase. Samples
148 for the biogas tests were taken during the course of 1 month, whilst the samples for the flocculation
149 tests were taken throughout a 6 months period. Considering the use of real wastewater and the
150 length of the experiments, results obtained can represent the effect of variability in used water
151 resources, thereby allowing inferring experimental results more representative to real systems.

152 **2.2 Flocculation and bioflocculation tests**

153 *2.2.1. Pre-testing of flocculation with different coagulants*

154 The coagulation aids included AlCl₃ (Sigma Aldrich), the cationic biopolymer Greenfloc 120
155 (Hydra 2002, Hungary) and the cationic polymer Poly(diallyldimethylammonium chloride)
156 (PDADMAC) (Sigma Aldrich). Jar testing was done based on the standard practice for coagulation-
157 flocculation jar test of water [29]. Each flocculation test included the parallel testing of six 1 L jars
158 using a mixing device with a rotating impeller mixing each jar. In each jar, a chosen coagulation aid
159 was spiked at varying concentrations, while mixing. During the first 2 min, a high mixing rate (150

160 rpm) was applied to evenly mix the added coagulants with the algae. This step was followed by a
161 slow mixing at 25 rpm to let the particles flocculate for 10 min. After the flocculation, the mixing
162 was stopped and the impellers were removed from the solution to initiate 30 min settling.

163 *2.2.2. Two-step flocculation*

164 Two flocculation methods were tested in 1 L jars: i) the bacterial biomass was used to flocculate
165 algae and ii) a two-step flocculation was tested where in the first step the algae were coagulated first
166 with the cationic polymer PDADMAC and then bacterial biomass was added in the second step to
167 enhance the flocculation. In the first case, high mixing was applied at 100 rpm for 2 min and
168 different slow mixing times (i.e. 10 min, 1 h and 3 h) were tested at 20 rpm. For the two-step
169 flocculation method the duration of flocculation is given in Table 1. Both methods were followed
170 by 30 min settling period.

171 **<Table 1>**

172 **2.3 Biomethane potential**

173 *2.3.1 Samples combinations for biomethane potential assays*

174 The settled biomass samples were collected after the two-step flocculation tests and kept at -20 °C
175 until further use. Additionally to the flocculated samples, microalgal and bacterial biomass were
176 collected to assess the biomethane potential (BMP) of the single biomasses and their combination
177 without polymer. All the samples were kept frozen until the BMP assays were set up. In total, eight
178 different scenarios were assessed in BMP assays using triplicates: algae, algae + polymer (20 mg
179 polymer/g algae), activated sludge (AS) alone (taken after the aerobic and anaerobic phase),
180 AS_{AE}/AS_{AN} + algae (ratio 0.1 of g algae/g AS) and lastly AS_{AE}/AS_{AN} + algae + polymer (ratio 0.1 of
181 g algae/g AS, 20 mg polymer/g algae). The SRT of the EBPR system was 3.5 days in all samples

182 used for BMP tests. The amount of substrate and inoculum as well as the total solids (TS) and
183 volatile solids (VS) concentrations of each sample are reported in Table S2, SI. The composition of
184 the substrates is reported in Table S3, SI.

185 *2.3.2 Biomethane potential assays set up*

186 The set up for the BMP assays was adapted from Angelidaki et al. [30]. Inoculum for the assay test
187 was taken from the mesophilic anaerobic digester of Lundtofte WWTP. The defrosted biomass
188 samples were added together with the inoculum to 1200 ml bottles, flushed with N₂ for 5 minutes,
189 closed with air tight rubber stoppers, sealed with screw caps and stored at mesophilic conditions at
190 37 °C. Avicel pH-101 was used as substrate for positive control and DI water as substrate for
191 negative control. The methane concentration produced in the bottles was measured every 2-3 days
192 using the GC-2010 (Shimadzu, Japan). On each measurement day a calibration curve was set up
193 using 5, 10, 40 and 60 % methane content to be able to relate the methane content of the samples.
194 Each time 50-100 µL sample was taken from the headspace using a pressure syringe and was
195 injected into the GC.

196 **2.4 Analytical methods and calculations**

197 The optical density (OD) at 750 nm was measured in the initial algae suspension and in the bacterial
198 biomass and was monitored during the 30 min settling by taking samples 5 cm below the liquid
199 surface (approximately at 700 ml in the 1 L jar) to maintain uniform sampling in all experiments
200 (adapted from [31]). In case the biomass blanket height was above 700 ml, due to poor settling of
201 bacterial biomass, the final OD sample at 30 min was taken from the supernatant above the biomass
202 height in order to calculate microalgal biomass recovery. OD samples were collected in 24 well
203 microplates and OD measurements were conducted in the end of each jar test using Synergy Mx

204 Microplate Reader® (Biotek). The recovery was calculated based on the following expression
205 (based on [32]):

$$206 \text{ Recovery (\%)} = \frac{OD_{750init} - OD_{750,30min}}{OD_{750init}} * 100 \quad \text{Eq. 1}$$

207 where $OD_{750init}$ is the OD of the initial suspension, $OD_{750,30min}$ is the OD measured at the end of the
208 settling phase. Average recovery and the standard deviation were calculated based on the last three
209 measurement points of the 30 min settling period.

210 The price of harvesting the microalgal biomass using different coagulants was calculated. The
211 estimations were based on the price of $AlCl_3$, Greenfloc 120 and PDADMAC reported by the
212 suppliers (see section 2.2.1) in 2014, when the experiments were conducted.

213 Total suspended solids (TSS), volatile suspended solids (VSS) and TS and VS of the algae and
214 bacterial biomass were measured based on standard methods [33]. Sludge volume index (SVI) of
215 the bacterial biomass was measured in 1L cylinder based on Ekama et al. [34]. Total nitrogen and
216 phosphorus and COD measurements in the samples were done using commercial test kits (Hach-
217 Lange®, USA) and measured with spectrophotometer DR2800 (Hach-Lange).

218 The average methane yield and the standard deviation were calculated based on triplicate batch tests
219 conducted for each scenario. Each replicate was collected on a different day as the amount of
220 bacterial and algal biomass was not enough for more than one flocculation test.

221 We calculated the methane yield produced during the co-digestion of algae and bacteria based on
222 Wang et al. [35]:

$$223 \text{ Calculated methane yield} = Y_s * C_s + Y_a * C_a \quad \text{Eq. 2}$$

224 where Y_s and Y_a is the measured methane yield of bacterial biomass and algae produced
225 individually and C_s and C_a is the mixing fraction of bacteria and algae in the co-digestion scenario.
226 These numbers were confronted with the measured methane yields of the co-digestion scenarios,
227 assessing the synergistic effect of co-digestion, and results are shown in section 3.3.

228 First-order kinetics is used to estimate the hydrolysis constant (k_h) and the ultimate methane
229 production (B_∞) based on Angelidaki et al. [30] and Ge et al. [36]:

$$230 \quad B = B_\infty(1 - e^{-k_h*t}) \quad \text{Eq. 3}$$

231 where B is the methane produced at a given time.

232 Student's t-tests were conducted, based on the triplicate samples, to compare the measured methane
233 yields for the different digestion scenarios, using SigmaPlot (USA).

234 **3. Results and discussion**

235 **3.1 Flocculation of microalgae using different coagulants**

236 $AlCl_3$ was effective for harvesting the microalgae, and dosing at 100 mg $AlCl_3$ /g algae resulted in a
237 recovery of 97% after 30 min settling time (Fig. 1a). A different trend in the recovery was obtained
238 when using polymers. The optimum Greenfloc 120 dosing was 30 mg GF/g algae, yielding 84%
239 recovery, based on visual observations (Fig. 1a). However, when a higher polymer concentration
240 was added to the suspension, the recovery decreased. This is the likely consequence of the
241 restabilisation process whereby increasing the amount of positive charges will result in repulsion
242 between the aggregates [21]. Similarly, when coagulation was induced by the addition of
243 PDADMAC an optimum recovery of 92% was found at the intermediary dose of ca. 27 mg
244 PDADMAC/g algae (Fig. 1a).

<Figure 1>

245

246 The optimal dosage of AlCl_3 is within the reported range for aluminium salts, 85 - 503 mg
247 aluminium salt/g algae [37,38]. The optimal cationic polymer dosage reported in the literature, (e.g.
248 Roselet et al. [21], 19.23 - 57.69 mg polymer /g algae) is in agreement with the range found in our
249 study. Restabilisation, as we observed, is not always reported: whilst some observed restabilisation
250 (e.g. [21,39]), others (e.g. [40]) found no restabilisation as the amount of polymer was increased.
251 Gerde et al. [31] observed restabilisation at lower biomass concentrations (0.05-0.2 g/L), whilst at
252 high biomass concentrations (1 g/L) this effect was not visible within the same dosing range. This
253 may be important when considering cultivation conditions and reactor operation. Depending on the
254 cultivation conditions, i.e. open ponds or closed photobioreactors, the biomass concentration during
255 the cultivation can vary from 0.1 – 4 g/L [41]. The maximum biomass concentration that can be
256 reached in open ponds and closed photobioreactors is 1 g/L and 4 g/L, respectively [42]. In this
257 study, the system resembles an open pond reactor with comparably low biomass concentration,
258 which may lead to algae restabilisation.

259 The optimum AlCl_3 dosage would result in a cost of approximately 6000 EUR/ton algae harvested
260 (Fig. 1b), whilst the use of Greenfloc 120 and PDADMAC at an optimal dose would be 30 – 60
261 times lower, about 100 and 900 EUR/ton algae, respectively (Fig. 1b). Moreover, the use of
262 aluminium salts may pose negative effects in terms of downstream recycling of the effluent water
263 [43] that can limit further usage of the biomass for land application or biogas production [44] due to
264 their substantial toxic effects [45]. However, according to Udom et al. [46] polymers have
265 substantial environmental and economic costs related to their production process. The greenhouse
266 gas emission and the energy consumption costs related to the production of polymers are found to
267 be nearly ten times higher than for ferric chloride [46]. Thus even though we save on the
268 operational costs due to the lower dosage, there are additional energy-expensive costs related to the

269 use of polymers. Recovery rates obtained with PDADMAC and Greenfloc are not significantly
270 different (based on t-test, $P>0.05$). Due to the similar performance and the easier access on the
271 market (Greenfloc had limited availability for research purposes) PDADMAC was chosen for
272 further assessment.

273 **3.2 Bioflocculation of microalgae – an innovative approach**

274 Flocculation of microalgae with bacterial biomass by 10 min flocculation time resulted in 40%
275 recovery (Fig. S2, SI). Furthermore, increasing the mixing time did not improve the microalgal
276 recovery (Fig. S2, SI), in contrast to the observations by Manheim and Nelson [26]. Alternatively,
277 we considered addition of cationic polymer as coagulation aid to destabilise the microalgae before
278 the addition of bacterial biomass and to enhance the separation of microalgae.

279 Different concentrations of polymer addition were tested (Fig. 2a). With increasing polymer
280 concentrations the microalgal recovery increased as well. This suggests that as the algal cells
281 aggregate into larger particles the probability of collision with the bacterial biomass flocs can
282 significantly increase, thereby increasing flocculation efficiency. No restabilisation effect was
283 observed at the assessed dosing, likely due to the high concentrations of bacterial biomass addition,
284 in accordance with the findings of Gerde et al. [31]. However, we *note* that there might be
285 restabilisation at higher polymer dosages [31]. Recovery rate ca. 97% was obtained using a polymer
286 dosage of 16 mg/g algae at a 0.1 g algae/g bacterial biomass ratio. Using bacterial biomass and
287 polymer for the coagulation-flocculation can reduce the polymer dosing by 40% compared to the
288 scenario when only algae was flocculated with the cationic polymer, PDADMAC (Fig. 2b).
289 Consequently, harvesting costs are reduced.

290 <Figure 2>

291 The mixing ratio was fixed at 0.1 g algae/g bacterial biomass for most experiments. With increasing
292 algae-to-bacterial biomass ratio, maintaining the same polymer dosage (16 mg polymer/g algae), the
293 microalgal recovery decreased, on average, with more than 50% (Fig. 3). This shows the
294 importance of assessing the optimum polymer dosing for the operational algal-to-bacterial biomass
295 mixing ratio. However, some deviation from the optimum ratio will not compromise the recovery as
296 we find similar recovery at 0.2 g algae/g bacterial biomass.

297 **<Figure 3>**

298 The flocculation efficiency of microalgae and the required dosing of coagulants and flocculants can
299 be influenced by factors, such as mixing time [26], pH [40] or the growth stage and age of the
300 microalgal culture [20]. Autoflocculation due to the increase of pH typically occurs above pH=10
301 [32,47]. Therefore, the effect of pH should be negligible as it was kept below 8.5 during the
302 experiments (section 2.1.2). We assume that the algae samples were in similar physiological state
303 for all flocculation experiments as the algal biomass was harvested every 2-3 days. Moreover, it is
304 reported in the literature that a certain concentration of inorganic coagulant can result in different
305 recovery for different microalgal species [21,48]. Thus in a mixed microalgal culture if the
306 dominance of the microalgal species changes the flocculation efficiency can potentially change.
307 However, microbial community was not monitored in this study. In addition, this effect can be
308 potentially compensated by the addition of the bacterial biomass as it can hinder the restabilisation
309 effect in the tested dosing range.

310 The settleability of the bacterial biomass varied in the EBPR system due to filamentous bulking,
311 which could have affected the observable flocculation efficiency. During the experiments, the SVI
312 (an indicator of the settling characteristics of the bacterial biomass [34]) varied between 180 and
313 760 ml/g, which allowed us to test the effect of bacterial biomass settling on the recovery of

314 microalgal biomass. The separation of the bacterial-microbial biomass after flocculation might be
315 limited if bulking (high SVI) bacterial biomass is used (Fig. S3, SI). Even though the separation of
316 the bacterial-algal biomass deteriorates, the recovery of microalgae is not affected by the increased
317 SVI of bacterial biomass (Fig. 4). Thus the bacterial composition has no particular effect on the
318 microalgal recovery. Additionally, the commonly believed particle screening effect of filamentous
319 bacteria, whereby filaments are the backbone of flocs, responsible for incorporating colloidal
320 particles into the floc structure [49] does not seem to play a significant role in the flocculation of
321 algal biomass. Instead, the surface charge of the biomass may control the flocculation behaviour.
322 The negative surface charge of the biomass comes in contact with the positive charges of the
323 polymer that is attached to the algae, thereby promoting aggregate formation. Despite the low
324 impact on microalgal recovery, from an operational perspective, the abundance of filamentous
325 organisms in bacterial biomass is an important factor, responsible for causing foaming in anaerobic
326 digesters, that could deteriorate digester performance [50].

327

<Figure 4>

328 **3.3 Co-digestion of algal and bacterial biomass**

329 The biomethane potential (BMP) obtained after 27 days of digestion of the microalgal biomass is
330 331 ± 76 ml CH₄/gVS (Fig. 5). The methane yield obtained by digesting solely microalgal biomass is
331 reported in a wide range in the literature (143-497 ml CH₄/gVS) [2,18], which also corresponds to
332 the results obtained in this study. Wang and Park [13] report slightly lower yields (230 ml
333 CH₄/gVS) when digesting *Chlorella sp.*, whereas Olsson et al. [51] report similar values when
334 digesting algae (mixed green microalgal culture – approx. 370 ml CH₄ /gVS). In this study, we
335 obtain similar methane yields without the pre-treatment of algae to those that are reported with
336 different pre-treatment options in the literature (105-336 ml CH₄ /gVS) [52]. Nevertheless,

337 Anbalagan et al. [53] showed that pre-treatment does not always result in higher BMP as, e.g., the
338 nutrient balance and type of algae are also important factors affecting the methane yield. In
339 addition, the variations reported through the literature might be due to the dominance of different
340 species in a mixed culture, which can affect the biogas potential [51]. The addition of polymer does
341 not significantly affect the biomethane potential of the microalgae.

342 **<Figure 5>**

343 The biomethane potential of the biomass removed after the aerobic phase is 363 ± 68 ml CH_4/gVS ,
344 whereas, for biomass removed after the anaerobic phase is 449 ± 17 ml CH_4/gVS (Fig. 5, Table 2).
345 The difference between these two digestion scenarios is not significant. Kuglarz et al. [54] reported
346 generally lower methane yields compared to our measurements when digesting bacterial biomass,
347 taken from a conventional wastewater treatment plant, even after pre-treatment (approx. 270 ml
348 CH_4/gVS). It is reported by Bolzonella et al. [55] that higher biogas potential is reached when
349 bacterial biomass is taken from shorter SRT (8 d in their study) wastewater treatment systems
350 compared to systems with longer SRT (45 d in their study). Literature is relatively scarce in regard
351 to assessing the biogas potential of short-SRT bacterial biomass. The study by Ge et al. [36] reports
352 similar results to those obtained with the biomass removed after the aerobic phase in our study
353 (BMP: $306.4 \pm 12.6 - 332.4 \pm 19.7$ ml CH_4/gVS). These BMP values are significantly lower than that
354 obtained using bacterial biomass collected after the anaerobic phase in the short-SRT EBP2R
355 process.

356 The hydrolysis rate and the ultimate biomethane potential were estimated by fitting Eq. 3 on the
357 data obtained during the 27-day long digestion tests (Table 2). The k_h for the anaerobic digestion of
358 microalgae found in this study is higher than those reported in the literature [56]. Only Ge et al. [36]

359 report k_h values ($0.19\pm0.02 - 0.22\pm0.04 \text{ d}^{-1}$) that are comparable to those obtained in this study with
360 aerobically and anaerobically harvested bacterial biomass.

361 <Table 2>

362 The co-digestion of the bacterial biomass removed after the aerobic phase and microalgal biomass
363 resulted in higher amount of methane produced than by digesting them individually (not
364 significantly different, $P>0.05$). Whereas, the co-digestion of algae with bacterial biomass collected
365 after the anaerobic phase resulted in significantly higher methane yields compared to digesting the
366 algal and bacterial biomass separately, based on the results of the t-test (Table S4, SI). Values of the
367 measured yield obtained with and without polymer are 424 ± 14 (Algae + AS_{AE} + poly) and 400 ± 22
368 (Algae + AS_{AE}), respectively, expressed as ml CH₄/gVS. This is approximately 10% higher than
369 that reported in the literature [51]. The calculated methane yield (Eq. 2) for the co-digestion
370 scenario with the bacterial biomass collected after the aerobic phase is 360 ± 62 ml CH₄/gVS (Table
371 2). We find no significant difference ($P>0.05$) between calculated (based on Eq. 2) and measured
372 values. Thus, our results suggest no synergistic effect when co-digesting algae and bacterial
373 biomass removed after the aerobic phase, in agreement with the literature [15]. The calculated
374 methane yield (Eq. 2) for the co-digestion scenario with the bacterial biomass collected after the
375 anaerobic phase is 437 ± 17 ml CH₄/gVS (Table 2). Values of the measured yield obtained with and
376 without polymer are 528 ± 28 (Algae + AS_{AN} + poly) and 560 ± 24 (Algae + AS_{AN}), respectively,
377 expressed as ml CH₄/gVS. Thus, we find that the measured values are significantly higher ($P<0.05$)
378 than the calculated values based on Eq. 2. These results suggest that – as opposed to using the
379 bacterial biomass removed after the aerobic phase – there may be synergistic effects of co-digesting
380 algae with biomass removed after the anaerobic phase, compared to digesting them individually.
381 Furthermore, the biomethane potential of the co-digestion was significantly higher ($P<0.05$) with
382 bacterial biomass taken after the anaerobic phase than with biomass taken from the aerobic phase

383 (Fig. 5, Table 2). The higher co-digestion potential with bacterial biomass removed after the
384 anaerobic phase could be related to their content of PHA. It is well known that PAO store VFA in
385 the form of PHA under anaerobic conditions [12] which is a more easily available substrate for the
386 digestion than other organic materials, e.g. the cell wall. Interestingly, we do not find significant
387 difference between the single digestion of biomass taken after the anaerobic and aerobic phase. This
388 suggests that the single digestion of the bacterial biomass may be nutrient limited, thereby
389 producing less methane. Whereas, co-digestion with a nutrient rich biomass, e.g. microalgae, could
390 provide the additional nutrients needed to digest the increased organic carbon content, resulting in
391 higher methane potential. Additionally, other studies suggest that the increased micronutrients
392 content added with the microalgal biomass can improve the biogas potential when co-digesting
393 algae with bacterial biomass [51]. Presence of the cationic polymer after the flocculation did not
394 affect the co-digestion potential (no significant effect, $P>0.05$), in agreement with the literature [23]
395 (Table 2).

396

<Figure 6>

397 It was estimated that 0.4 ± 0.02 g $\text{COD}_{\text{CH}_4}/\text{g COD}_{\text{inf}}$ and 0.36 ± 0.07 g $\text{COD}_{\text{CH}_4}/\text{g COD}_{\text{inf}}$ energy can
398 be recovered in the form of methane, for the anaerobic and aerobic bacterial biomass removal
399 scenarios, respectively. These results are considerably higher than that obtained for conventional
400 activated sludge systems (0.07 ± 0.06 g $\text{COD}_{\text{CH}_4}/\text{g COD}_{\text{inf}}$) and are comparable to other short-SRT
401 activated sludge systems (0.36 ± 0.08 g $\text{COD}_{\text{CH}_4}/\text{g COD}_{\text{inf}}$) [57]. The assessment of the distribution
402 of the influent COD (Fig. 7) shows that not only approximately 40% of the influent COD is
403 recovered as methane, but the EBPR system effectively removes most of the influent COD leaving
404 up to maximum 10% as inert material in the effluent wastewater. Compared to other short-SRT
405 systems, these results show significantly lower loss of COD in the effluent while directing
406 comparable amounts into the biomass [9,57,58]. This facilitates downstream unit process operation,

407 e.g. microalgal cultivation or autotrophic nitrogen removal based technologies. Taken together, our
408 results suggest that there is an increased methane potential of the co-digestion of bacterial biomass
409 generated through the short-SRT EBPR system and microalgal biomass. Furthermore, the
410 associated environmental costs are lower as pre-treatment of the biomass is not necessary and less
411 energy is invested for pollutant removal compared to systems with long solid retention times.
412 Moreover, the COD recovered through the EBPR process is comparable to that found in the
413 literature for other short-SRT systems, leaving up to maximum 10% as inert material in the effluent
414 water.

415 <Figure 7>

416 **4. Conclusions**

417 In this study we assessed an innovative bioflocculation method to harvest microalgal biomass and
418 evaluated the potential to produce methane through digestion and co-digestion of the recovered
419 microalgal biomass with bacterial biomass derived from an EBPR system. We found that:

- 420 • The cationic polymer (PDADMAC) proved to be a cost-efficient way to harvest microalgal
421 biomass resulting in 92% recovery with 27 mg polyelectrolyte/g algae dosing.
- 422 • An innovative bioflocculation method was introduced to separate microalgal biomass. Bacterial
423 biomass was used as a flocculant after the destabilization of microalgae with cationic polymer,
424 whereby up to 97 % recovery was reached with 16 mg polymer /g algae and 0.1 g algae/g
425 bacterial biomass ratio.
- 426 • The highest methane yield was found at 560 ± 24 mlCH₄/gVS when microalgae and
427 anaerobically harvested bacterial biomass were co-digested.
- 428 • The short-SRT EBPR process combined with microalgal cultivation can serve as an energy
429 recovery system whereby up to 40 % of the incoming COD is converted to methane through

430 anaerobic digestion. Moreover, the COD is successfully removed through the process, thereby
431 leaving only up to 10% inert COD in the effluent wastewater. However, the optimization of the
432 nutrient balance during the anaerobic digestion by co-digestion with nutrient rich biomass, e.g.
433 microalgae, is important to potentially increase the COD recovery.

434 **Acknowledgements**

435 Dorottya S. Wágner thanks the European Commission (E4WATER Project, FP7-NMP-2011.3.4-1
436 grant agreement 280756) for the financial support. Borja Valverde-Pérez thanks the Integrated
437 Water Technology (InWaTech) project (<http://www.inwatech.org>) for the financial support. The
438 authors thank Kirska Nørregaard for the help in conducting some of the experiments.

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