

1 **IMPROVING THE ENERGY BALANCE OF AN INTEGRATED**
2 **MICROALGAL WASTEWATER TREATMENT PROCESS**

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9 **Keywords:** microalgae, harvesting, cell wall, thermal hydrolysis, anaerobic digestion, energy
10 balance.

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12 **Waste and Biomass Valorization DOI: 10.1007/s12649-013-9230-2**

13 **Abstract**

14 The inclusion of a microalgal system in a wastewater treatment flowsheet for residual
15 nutrient uptake can be justified by processing the waste biomass for energy recovery. Low
16 energy harvesting technologies and pre-treatment of the algal biomass are required to
17 improve the overall energy balance of this integrated system. *Scenedesmus obliquus* and
18 *Chlorella* sp., achieving nitrogen and phosphorus removal rates higher than 90%, were used
19 to compare cells recovery efficiency and energy requirements of two energy efficient
20 harvesting systems: Dissolved Air Flotation (DAF) and Ballasted Dissolved Air Flotation
21 (BDAF). In addition, thermal hydrolysis was used as a pre-treatment to improve biogas
22 production during anaerobic digestion. The energy required for both systems was then
23 considered to estimate the daily energy demand and efficiency of two microalgae wastewater
24 treatment plants with a capacity of 25,000 and 230,000 p.e., respectively. Overall, a high
25 algal cells recovery efficiency (99%) was achieved using low energy demand (0.04 kWh m⁻³
26 for BDAF) and a coagulant dose reduction between 42 and 50% depending on the algal
27 strain. Anaerobic digestion of pre-treated *S. obliquus* showed a 3-fold increase in methane
28 yield. Compared to a traditional activated sludge process, the additional tertiary microalgal
29 treatment generates an integrated process potentially able to achieve up to 76% energy
30 efficiency.

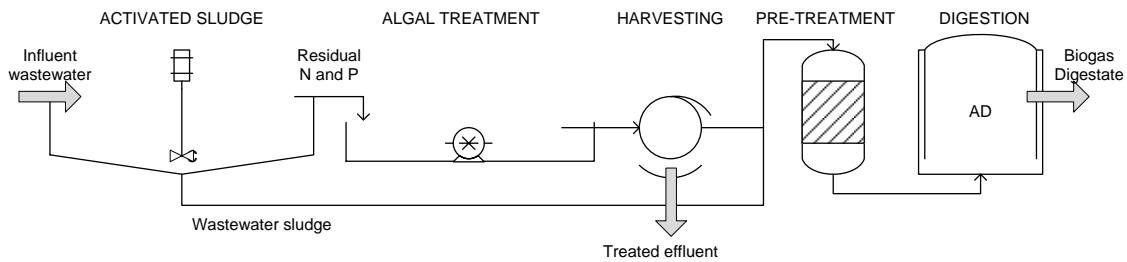
31 INTRODUCTION

32 Utilisation of algae as part of a nutrient removal strategy within wastewater treatment enables
33 relatively passive polishing of residual nitrogen and phosphorus in reactors with residence
34 times ranging between 2-4 days [1,2]. For instance, batch reactors operated over a 2-day
35 cycle time containing *Chlorella vulgaris* and *Scenedesmus obliquus* resulted in 80 and 96%
36 removal of ammonia respectively [3]. Extending residence times to 15 days with *S. obliquus*
37 has demonstrated the capability to reach effluent concentration as low as 0.01 mg l⁻¹ total
38 phosphorus (TP) [2] indicating potential for small works to meet very low discharge consents
39 as long as sufficient land is available. In addition to nutrient removal, microalgae can acts as
40 CO₂ sequestration agent at rates of around 1.8 kg_{CO2} kg_{biomass}⁻¹ and so have the potential to be
41 integrated into biogas upgrade loops as a means of CO₂ disposal.

42 A range of reactor configurations have been considered including algal ponds, photo-
43 bioreactors, immobilisation and attached systems [4,5,6]. Photo-bioreactors are typically used
44 when high value products are generated from the algae biomass where concentrations in the
45 reactors can reach up to 2 kg m⁻³ [7,8]. In the case of wastewater treatment the majority of
46 systems are based on algal ponds where biomass concentration remains below 1 kg m⁻³ with
47 average values between 0.2 and 0.6 kg m⁻³ [8,9]. In either configuration two additional
48 requirements must be met for them to be integrated into a wastewater treatment flowsheet.
49 Firstly, the algae must be separated from the water phase prior to discharge and secondly the
50 algae must be used/disposed of. In the wastewater context, anaerobic digestion of the
51 collected biomass appears the most sensible option as the quantities are generally quite small
52 and the AD assets already exist. In such cases an interesting opportunity presents itself
53 whereby the energy required to operate the algae reactors may be offset by the additional
54 energy produced through digestion of the used algal biomass. Examination of the
55 requirements for integration of algae reactors into a standard wastewater flowsheet reveals
56 two key components: (i) the need for a low energy cell recovery system to reduce energy
57 requirement for biomass harvesting and (ii) the need to maximum biogas production from
58 algae through pre-treatment of the algal cells.

59 Typical separation processes used for algae harvest include centrifuges or pressure and
60 vacuum filters with associated energy demands ranging between 0.3 and 8 kWh m⁻³ [10]. At
61 large scale, low energy systems (< 0.3 kWh m⁻³) such as chemical flocculation, bio-
62 flocculation or autoflocculation, are considered efficient pre-concentration technologies

63 which can reduce operation costs when combine with traditional harvesting system [11,12].
64 The main alternative to those is the use of dissolved air flotation (DAF). The system
65 generates micro bubbles of air which attach to algae cells and allow them to float [13].
66 Generation of the bubble is through released of a supersaturated water solution akin to beer
67 production and has an energy associated with it of around 0.3 kWh m⁻³ [14]. Recent
68 innovations in the technology have replaced the produced air with glass beads in a process
69 called ballasted dissolved air flotation (BDAF) were the beads can be recycled enabling
70 reduction in energy of 60-80% compared to traditional DAF systems [14]. Anaerobic
71 digestion of algae in traditional mesophilic digesters yields between 30 and 50% of the
72 potential theoretical values [15,16,17]. Higher efficiencies has been reported for thermophilic
73 conditions or when co-digesting algae with other biomass [18,19]. In all cases, the hardness
74 of the cell wall seems to represent the main inhibitor factor [17,20]. The cell wall of green
75 algae is mainly composed of sugars (24-74%), such as glucose, mannose and galactose,
76 forming cellulose and hemicellulose with biopolymers (e.g. sporopollenin, algaenan) which
77 are responsible of the thickness and the resistance of the cells to bacteria degradation [21,22].
78 In order to overcome this limitation, a range of pre-treatment methods such as ultrasound,
79 high temperature, French press and enzymes have been used to improve algae digestion and
80 biomethane yields [21,23,24]. In relation to wastewater treatment, one of the most commonly
81 used pre-treatment processes is the thermal hydrolysis [25,26]. The process works by
82 applying a combination of temperature (150-170°C) and pressure (6-8 bar), which breaks
83 down the physical structure of all the organic material including algae.
84 Linking together the innovative approaches outlined here potentially improves the
85 opportunity to be more sustainable and energetically balanced in relation to nutrient removal.
86 The current paper considers this by evaluating the impact of inclusion of these technologies
87 in a wastewater flowsheet containing an algal reactor for nutrient polishing (Figure 1). In
88 particular the work compares Dissolved Air Flotation and Ballasted Dissolved Air Flotation
89 for algae collection and the effect of a thermal hydrolysis pre-treatment on algal cell
90 disruption and digestion yields using *S. obliquus* and *Chlorella* sp. The two technologies
91 were combined in different scenarios to estimate the energy demand and the energy
92 efficiency at two different scales of operation: 25,000 and 230,000 p.e., respectively.



93
94 Figure 1: Schematic diagram of an integrated microalgae wastewater treatment process

95 MATERIALS AND METHODS

96 *Algal culture*

97 Experiments were conducted on two single cell green microalgae species: *S. obliquus*
98 (276/42) and *Chlorella* sp. (211/BK) which were obtained from the Culture Collection for
99 Algae and Protozoa (Oban, UK) and cultivated in Jaworski Media [27]. Algal growth was
100 characterised using cell counting with soluble protein content (sPC) and soluble carbohydrate
101 content (sCC) measured according to the methods described by Henderson et al. [27]. Solids
102 content, chemical oxygen demand (COD) and soluble COD (sCOD) were measured
103 according to APHA standard methods [28].

104 *Microalgae harvesting*

105 Jar tests experiments (11) were undertaken using an EC Engineering DBT6 DAF jar tester
106 (Alberta, CND). The DAF and BDAF tests were performed according to Henderson et al.
107 [27] and Jarvis et al. [14], respectively. Biomass concentration of $5 \times 10^6 \pm 10^5$ cells ml⁻¹ was
108 used for the different testing condition. The pH was adjusted to 7 using a 0.1 M HCl and 0.1
109 M NaOH solution. 300 mg l⁻¹ of low-density glass beads between 40 and 100 µm with a
110 density of 100 kg m⁻³, from Trelleborg Emerson and Cuming Inc. (Mansfield, USA) were
111 used after a pre-flotation test to eliminate non-floating beads [14]. Aluminium sulphate
112 (Al₂(SO₄)₃) was used as coagulant. The clarified samples were analysed for residual cell
113 content. All analyses were carried out in duplicate.

114 *Thermal hydrolysis treatment*

115 Thermal hydrolysis of the algal biomass was achieved using a Baskerville autoclave and
116 steam generator WON15827 (Manchester, UK). The unit is composed of two connected
117 pressure vessels. Steam generated at 165°C and 8 bar was flash-injected for 30 min into the
118 reaction vessel where concentrated algae, 200 ml at 2.0 ± 0.5 g TS l⁻¹, were maintained at

119 90°C. Cell counting, solid content, COD, sCOD, sCP, sPC were measured in duplicate before
120 and after treatment.

121 *BioMethane Test (BMT)*

122 The biomethane production was determined using a modified method of Angelidaki et al.
123 [29]. Digested sludge seed (inoculum) was obtained from a local WWTP and incubated at
124 38°C for 2-3 weeks to eliminate any residual activity. Seed and pre-concentrated algal
125 biomass were mixed to obtain a volatile solids (VS) ratio of 1:1 ($VS_{seed}:VS_{algal}$). 20 ml of the
126 mix was then transferred to a 100 ml serum bottle. The pH was adjusted to a value of 7 using
127 a 1 M NaOH solution and the bottles were filled with 40 ml of nutrient solution [29] to a final
128 volume of 60 ml leaving a head space of 40 ml. All tests were flushed with N₂ gas, sealed
129 with a PTFE crimp cap, and then placed into a shaking incubator at 38°C and 150 rpm.
130 Biogas production and composition were determined at day 2, 5, 8, 12, 16, 21 and 25. The
131 methane content was measured using a Servomex 1440 gas analyser (Crowborough, UK). All
132 tests were conducted in triplicate using treated or untreated algae.

133 *Energy efficiency evaluation*

134 The daily energy demand and efficiency of an integrated wastewater plant involving an
135 activated sludge (AS) system followed by a microalgal raceway pond and an on-site
136 anaerobic digestion (AD) was evaluated using the data reported by Shi [25] and by Zamalloa
137 et al. [30]. The efficiency of the WWTP is defined as the percentage amount of energy
138 produced compare to the total energy demand. The treatment capacity and energy
139 requirement of the two plant sizes considered are shown in Table 1. Six different scenarios
140 with different configuration were considered including:

- 141 1. Activated Sludge and Anaerobic Digestion (AS+AD)
- 142 2. Activated Sludge, Algal Pond, DAF harvesting system and Anaerobic Digestion
143 (AS+Pond+DAF+AD)
- 144 3. Activated Sludge, Algal Pond, BDAF harvesting system and Anaerobic Digestion
145 (AS+Pond+BDAF+AD)
- 146 4. Activated Sludge and Anaerobic Digestion with a Pre-Treatment step (AS+Pre-treat.+AD)
- 147 5. Activated Sludge, Algal Pond, DAF harvesting system and Anaerobic Digestion with a
148 Pre-Treatment step (AS+Pond+DAF+Pre-treat.+AD)
- 149 6. Activated Sludge, Algal Pond, BDAF harvesting system and Anaerobic Digestion with a
150 Pre-Treatment step (AS+ Pond+BDAF+Pre-treat.+AD).

151 Harvesting energy demand values used were equivalent to 0.3 kW m^{-3} and 0.04 kW m^{-3} for
 152 DAF and BDAF system, respectively [14]. The energy generated by the wastewater sludge
 153 digestion was back calculated from the assumed energy efficiency (Table 1). Additional
 154 energy from algal digestion was estimated using the methane yields reported in the present
 155 work, applying a methane energy conversion of 9.7 kWh m^{-3} and 30% efficiency [25].

156 Table 1: Integrated WWTP parameter design

<i>Traditional WWTP (AS+AD)</i>	TP25K	TP230K
Capacity	25000 p.e.	230000 p.e.
Influent _a	$4200 \text{ m}^3 \text{ d}^{-1}$	$38640 \text{ m}^3 \text{ d}^{-1}$
Energy demand (AS) _b	0.6 kWh m^{-3}	0.45 kWh m^{-3}
Energy efficiency without AD pre-treatment _c	25%	35%
Energy efficiency with AD pre-treatment _c	40%	60%
<i>Algal treatment</i>	TP25K	TP230K
Pond dimension _d	8.4 ha	77.3 ha
Biomass production (VS) _e	1.06 ton d^{-1}	9.74 ton d^{-1}
Energy demand (cultivation) _f	$32.5 \text{ kWh ha}^{-1} \text{ d}^{-1}$	$32.5 \text{ kWh ha}^{-1} \text{ d}^{-1}$

157 a) water availability of 210 l d^{-1} p.e. and a recovery coefficient of 0.8; b) electricity consumption [25]; c) assuming a thermal hydrolysis
 158 energy demand of $30 \text{ Wh pe}^{-1} \text{ d}^{-1}$ [26]; d) raceway pond with 4 d HRT [2] and 0.2 m depth [30] e) biomass concentration of 280 g VS m^{-3} and
 159 an harvesting recovery coefficient of 0.9 for both *S. obliquus* and *Chlorella* sp.; f) electricity consumption of a low level mixing system
 160 (paddle wheel) to guarantee a velocity 15 cm s^{-1} [30].
 161

162 RESULTS AND DISCUSSION

163 *Harvesting technologies*

164 Full algal cells recovery (>99%) was achieved using both harvesting systems: BDAF
 165 confirming the potential to use a lower energy alternative to traditional DAF. The associated
 166 energy saving of using BDAF as opposed to DAF was estimated at 0.26 kW m^{-3} resulting in
 167 an overall reduction in energy of 0.98 MWh d^{-1} at the small scale and 9.04 MWh d^{-1} at the
 168 larger scale (Table 3). An additional benefit of using the BDAF configuration was observed
 169 in association to chemical usage with a 40% reduction in metal coagulant use at the operating
 170 pH of 7 with *S. obliquus* and 50% lower with *Chlorella* sp (Table 2). The difference in
 171 coagulant demand between *S. obliquus* and *Chlorella* sp. relates to differences in the AOM
 172 (Allogenic Organic Matter) composition for the two algae [31] with the reduced charge
 173 density associated with the AOM produced from *Chlorella* sp. requiring less coagulant for
 174 optimal removal. It was estimated that the BDAF allows coagulant saving up to 100 g
 175 $\text{Al}_2(\text{SO}_4)_3 \text{ m}^{-3}$ of influent water depending on the algal species. This reduction could represent

176 an economic saving of 525 € d⁻¹ at small scale, and 4057 € d⁻¹ at larger scale, based on the
 177 current average market price [32] of the aluminium salts.

178 Table 2: Cells recovery, energy input and coagulant dose required (mean ± SD) for DAF and
 179 BDAF.

	Cell recovery %	Coagulant dose mg Al l ⁻¹	Energy input* kW m ⁻³	Coagulant dose mg Al l ⁻¹	Energy input* kW m ⁻³
	DAF			BDAF	
<i>S. obliquus</i>	99	40 ± 14	0.3	23 ± 9	0.04
<i>Chlorella</i> sp.	99	8 ± 2	0.3	4 ± 1	0.04

180 * According with Jarvis et al. [14].

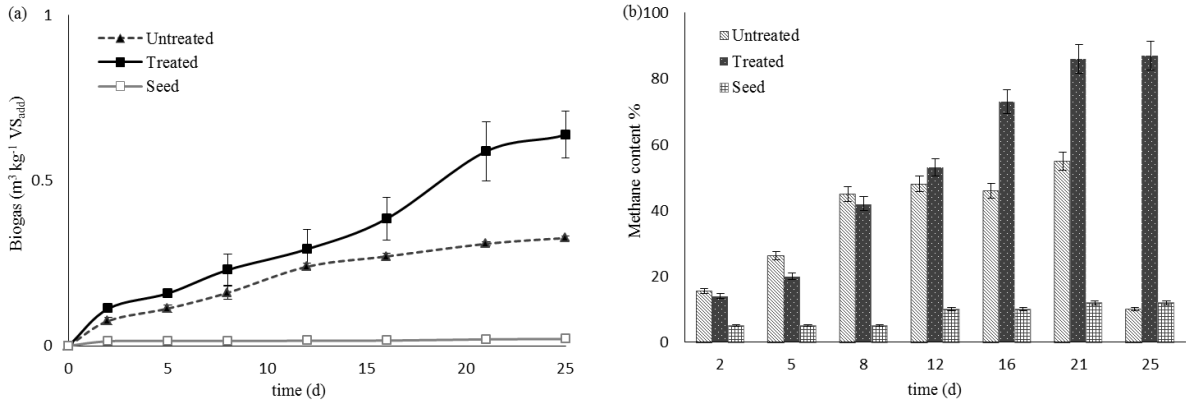
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182 *Thermal hydrolysis of the algal biomass*

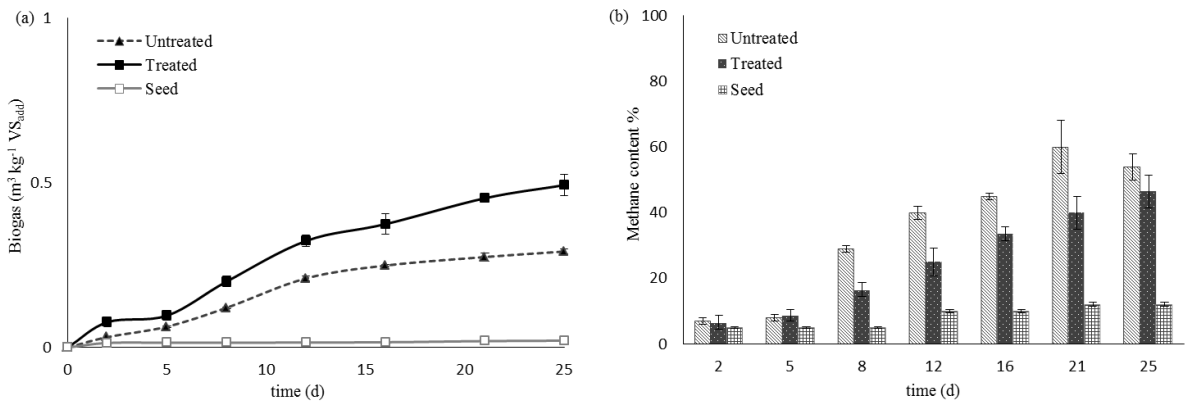
183 Thermal hydrolysis has a significant impact on the properties of the algal biomass of both
 184 species. To illustrate, in the case of *S. obliquus* the ratio between volatile suspended solids
 185 and volatile solids (VSS/VS) of the concentrated biomass decreased from 0.8 ± 0.2 to 0.5 ±
 186 0.2 as a result of pre-treatment. Whereas, in the case of *Chlorella* sp. the VSS/VS ratio
 187 decreased from 1 ± 0.2 before treatment to 0.8 ± 0.1 after treatment indicating a greater
 188 resistance to the impact of elevated temperatures and pressures. Microscopic analysis
 189 supported the observation of a difference in impact due to species selection based on the
 190 percentage of cells disrupted which decreased from 70 % in the case of *S. obliquus* to less
 191 than 50 % in the case of *Chlorella* sp. In addition, both algae showed post treatment
 192 aggregates as a consequence of releasing high amount of intracellular molecules which
 193 suggest that cells wall was disrupted [21]. The impact of these differences in terms of the
 194 released organic material were most noticed in terms of proteins where application of the pre-
 195 treatment step increase the level of soluble proteins from 25 mg l⁻¹ to 8149 mg l⁻¹ in the case
 196 of *S. obliquus* compared to an increase from 27 mg l⁻¹ to 2722 mg l⁻¹ in the case of *Chlorella*
 197 sp. A smaller level of difference was observed as a function of algal type in the case of
 198 soluble COD, which increased by 7528 mg l⁻¹ and 5306 mg l⁻¹ for *S. obliquus* and *Chlorella*
 199 sp. respectively. Whereas more similar changes in soluble carbohydrates were observed at
 200 2018 mg l⁻¹ for *S. obliquus* and at 2137 mg l⁻¹ for *Chlorella* sp.

201 The impact of this greater release of soluble material in the case of *S. obliquus* is observed in
 202 relation to the BMT (anaerobic digestion for 25 days at 38°C) where application of a pre-
 203 treatment step increased the methane yield from 0.13 ± 0.02 m³ kg⁻¹ VS_{add} to 0.32 ± 0.05 m³
 204 kg⁻¹ VS_{add}. This value is closer to the range of the theoretical methane content (0.53 - 0.54 m³
 205 kg⁻¹ VS_{add}) as calculated by Heaven et al. [16]. Untreated *Scenedesmus* biomass was reported

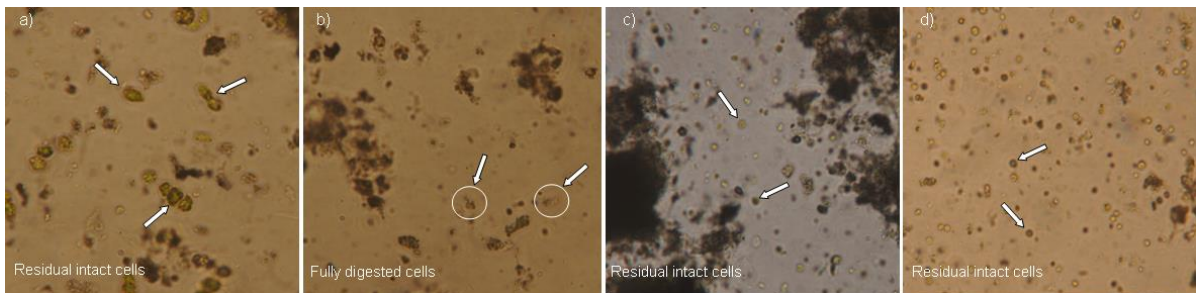
206 to yield between 0.12 and 0.18 m³ kg⁻¹ VS_{add} [15,21]. Our results compare favourably to the
207 one reported by Gonzalez-Fernandez et al. [21] who obtained a methane production of 0.22
208 m³ kg⁻¹ VS_{add} (133 dm³ kg⁻¹ COD_{in}) after thermal treatment at 90°C for 3h. Similarly, Alzate
209 at al. [24] achieved a final methane production of 0.36 and 0.40 m³ kg⁻¹ VS_{add}, closer to our
210 values, digesting a mixed culture (10 gTS kg⁻¹, 20% *Scenedesmus* sp.) after treatment at
211 140°C (1.2 bar) and 170°C (6.4 bar), respectively. The equivalent trial with *Chlorella* sp.
212 generated only a small change in methane yield, from 0.10 ± 0.01 to 0.15 ± 0.01 m³ kg⁻¹
213 VS_{add}, suggesting lower impact of the combined heat and pressure treatment on the cell wall.
214 Theoretical methane conversion values for this algae range between 0.45 and 0.57 m³ kg⁻¹
215 VS_{add} [16]. Different authors [15,33] reported higher methane yields than the one reported in
216 this paper digesting untreated *Chlorella* biomass (0.15 - 0.35 m³ kg⁻¹ VS_{add}). However, our
217 biogas yields were similar to the one reported in literature and increased from 0.29 ± 0.01 to
218 0.49 ± 0.03 m³ kg⁻¹ VS_{add} after the pre-treatment (Figure 3). The lower methane yields
219 obtained suggest a potential inhibition of the methanogenesis process, probably related to the
220 chemical composition of the algal biomass. Moreover, the thermal pre-treatment of *Chlorella*
221 sp. released a similar amount of carbohydrates and COD, but less proteins than *S. obliquus*.
222 These differences have generated different C:N ratios in the two systems which, as reported
223 by other authors [20], could have affected the overall biogas composition. This is confirmed
224 by the methane content in the biogas which decreased from 60 to 51% after pre-treatment
225 with *Chlorella* sp. (Figure 3b), while increased from 46 to 73% with *S. obliquus* (Figure 2b).
226 Microscopic observations of the digested samples showed no residual intact cells only for
227 pre-treated *S. obliquus* (Figure 4b). In all the other cases, residual algal biomass was
228 identified in the residual solids after digestion (Figure 4a, 4c and 4d) indicating the pre-
229 treatment had not sufficiently enhanced digestion of *Chlorella* sp. The results presented here
230 are in agreement with Valo et al. [34], who demonstrated that thermal hydrolysis pre-
231 treatment of a specific biomass (waste activated sludge) resulted in enhanced biogas
232 production and methane yield due to a reduction of the solids content and a parallel increase
233 of organic compounds released. However, the current work identifies that in the specific case
234 of microalgae the impact is likely to be highly related to a given algal species. The
235 differences are likely to be due to the thickness and composition of the cell wall, which is
236 known to vary between species [15,20].



237
238 Figure 2: *S. obliquus* BMT cumulative biogas production (a) and percentage methane content
239 (b) of treated and untreated algal biomass at 38°C.



240
241 Figure 3: *Chlorella sp.* BMT cumulative biogas production (a) and percentage methane
242 content (b) of treated and untreated algal biomass at 38°C.
243



244
245 Figure 4: Microscope analysis of digested sample (optical microscope x40); a) *S. obliquus*
246 untreated, b) *S. obliquus* treated, c) *Chlorella sp.* untreated, d) *Chlorella sp.* treated.
247

248 *Energy balance*

249 Anaerobic digestion of the collected sludge generates 25% and 35% of the total energy
250 demand required to run the works for the control case (scenario1, no algae, no pre-treatment)
251 for the small and the large scale respectively (Table 3). The remaining difference
252 demonstrates the importance of sludge imports on the overall energy balance on operating

253 sites. Generating additional solids for anaerobic digestion through the algal reactors, a
254 possible alternative to sludge imports, (scenario 2) resulted in an increase of the overall net
255 energy demand of the works by 61 and 95% for the small and large cases for both algal types.
256 The increase was a result of the energy required to operate the pond and DAF units not being
257 offset by the increased energy production. Adoption of the innovative BDAF process
258 (scenario 3) reduced this impact with an increase in net energy demand of only 9 and 14.5%.
259 These levels are similar to those of other tertiary nutrient removal processes which suggest
260 algal reactors may be suitable for use on an energy basis even with low biogas yields. For
261 instance, the energy values related to alternative tertiary treatments, such as wetlands (0 –
262 0.21 kWh m⁻³) [35] always required additional energy demand and do not produce a valuable
263 feedstock.

264 Inclusion of a sludge pre-treatment device in the non-algal case (scenario 4) resulted in an
265 increase in energy production of 0.68 MWh d⁻¹ at the smaller scale and 8.48 MWh d⁻¹ at the
266 larger scale. The additional energy production resulted in an increase in the net energy
267 balance across the works whereby the site produced 40 and 60% of the total demand at the
268 small and large scale respectively. The increased energy production from inclusion algae into
269 the pre-treated sludge mix (scenario5) enabled a greater proportion of the increased energy
270 demand from inclusion of the pond and the DAF unit to be met at both scales. To illustrate,
271 inclusion of an algae nutrient process decreased the overall energy efficiency of the works to
272 36% at the smaller scale using *Chlorella* sp., a decrease of 4% compared to the pre-treated
273 sludge only case (Figure 5, scenario 4). At the larger scale, the energy efficiency decreased
274 by 12% to a total value of 48% of the works demand due to the limited impact of the pre-
275 treatment on the algal methane production. In comparison, *S. obliquus*, which showed higher
276 energy production after pre-treatment, reported a 4% energy efficiency improvement at small
277 scale. However, at large scale the overall efficiency decreased from 60 to 57%. Switching to
278 the BDAF unit for harvest, changed the balance significantly. In the case of the small works
279 an increase in net energy demand of 0.12 MWh d⁻¹, compare with the control case, was
280 observed only considering *Chlorella* sp., although this included the entire energy demand of
281 the pre-treatment unit and so generated the lowest energy option in total. This was further
282 magnified at the larger scale where the increased energy generation from the pre-treated algal
283 biomass more than offset the energy demand of the pond, BDAF and pre-treatment units
284 leading to a net energy gain of 4.48 MWh d⁻¹ for *S. obliquus* and 1.08 MWh d⁻¹ for *Chlorella*
285 sp. In this case the energy production from biomass generated on site (sludge and algae) was

286 able to meet 76 and 64% of the total demand for energy, which represents an increase of 16
 287 and 4 % over the sludge only case (scenario 4).

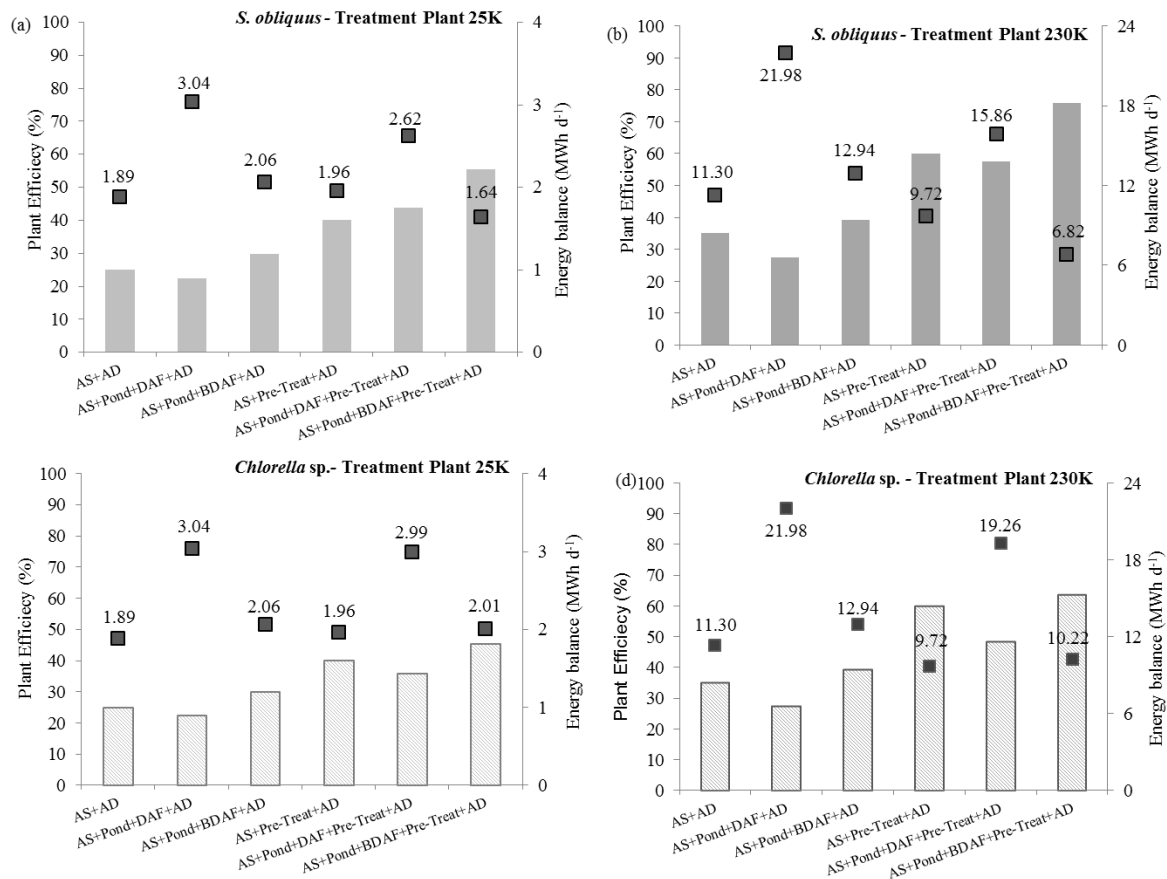
288 Table 3: Energy demand and efficiency of different integrated WWTPs configurations for
 289 both treatment plants and algal strains.

Scenarios description		Energy _a (MWh d ⁻¹)							
		Energy demand				<i>S. obliquus</i>		<i>Chlorella</i> sp.	
		Activated Sludge	Algae Pond	Algae Harvest	Sludge/Algae Pre-treat.	AD Energy recovery	Net energy consumption	AD Energy recovery	Net energy consumption
TP 25K	1 AS+AD	2.52				-0.63 _b	1.89 _b	-0.63 _b	1.89 _b
	2 AS+Pond+DAF+AD	2.52	0.27	1.13		-0.88	3.04	-0.88	3.04
	3 AS+Pond+BDAF+AD	2.52	0.27	0.15		-0.88	2.06	-0.88	2.06
	4 AS+Pre-treat.+AD	2.52			0.75	-1.31 _b	1.96 _b	-1.31 _b	1.96 _b
	5 AS+Pond+DAF+Pre-treat.+AD	2.52	0.27	1.13	0.75	-2.05	2.62	-1.68	2.99
	6 AS+Pond+BDAF+Pre-treat.+AD	2.52	0.27	0.15	0.75	-2.05	1.64	-1.68	2.01
TP 230K	1 AS+AD	17.39				-6.09 _b	11.30 _b	-6.09 _b	11.30 _b
	2 AS+Pond+DAF+AD	17.39	2.51	10.43		-8.35	21.98	-9.65	21.98
	3 AS+Pond+BDAF+AD	17.39	2.51	1.39		-8.35	12.94	-9.65	12.94
	4 AS+Pre-treat.+AD	17.39			6.90	-	9.72 _b	-14.57 _b	9.72 _b
	5 AS+Pond+DAF+Pre-treat.+AD	17.39	2.51	10.43	6.90	-21.37	15.86	-20.24	19.26
	6 AS+Pond+BDAF+Pre-treat.+AD	17.39	2.51	1.39	6.90	-21.37	6.82	-20.24	10.22

290 a) positive numbers represent electricity consumption values while negative numbers show electricity produced; b) the value reported for
 291 scenarios 1 and 4 represent energy generated from wastewater sludge digestion. In all the other scenarios the value shows the energy
 292 generated from algae/sludge co digestion by adding the two estimated energy values;
 293

294 Overall, the results demonstrate that when appropriate choices are made around the ancillary
 295 equipment then the use of algae for nutrient removal can represent a viable source of energy
 296 production and hence provide an energy neutral nutrient removal strategy. Critical to this is
 297 the use of pre-treatment to ensure the inclusion of algae in the anaerobic digestion generates
 298 sufficient biogas to justify its inclusion. In such case algae could be viewed as an appropriate
 299 alternative to co-digestion of imported non-sewage sludge wastes. The importance of this is
 300 that it avoids logistic and regulatory barriers and it enhances biogas production in digesters
 301 meant for sewage sludge processing. However, pre-treatment alone is insufficient as the
 302 energy demand of traditional technologies for algal separation is likely to be too high to
 303 justify the approach. In such case the significance of BDAF system becomes more important
 304 as it lowers the total energy demand by 1 MWh d⁻¹ at small scale and 9 MWh d⁻¹ at larger
 305 scale compared to the traditional DAF system. Ultimately both components are required to
 306 enhance the potential for inclusion of algae as a nutrient removal process.

307



308

309 Figure 5: Plant efficiency (column) and Energy balance (square) of the scenarios: *S. obliquus*
 310 in TP 25K (a) and TP 230K (b); *Chlorella* sp. in TP 25K (c) and TP 230K (d).
 311

312 The impact of which algae are used within the nutrient removal process was demonstrated in
 313 this study by looking at two similar single cell green algae both of which are commonly used
 314 in algal biomass production. In the current case an 8-9% difference was seen on the overall
 315 balance as a function of species with *Chlorella* sp. generating less energy than *S. obliquus*.
 316 The difference is thought to occur due to the combination of the strong species-specific wall
 317 structure found within *Chlorella* sp. [20,36] and the differences within the AOM generated
 318 and released after pre-treatment, effecting the final biogas composition. Given that the
 319 structure of the two algae strains is reasonably similar it is reasonable to assume that when
 320 using other algae species significantly different outcomes may occur. Common algae species
 321 found in the UK include filamentous strains of green, diatoms and blue-greens all of which
 322 have examples of appendages and mobility associated to them [37]. Previous work on
 323 separation of algae has shown that such differences can have a significant impact on the
 324 chemical and energy requirements for harvesting [31,38]. In addition previous studies on
 325 different algae have also shown species-specific outcomes in relation to the impact of pre-

326 treatment and the biogas production achieved [15,23,24]. Importantly the selection of the
327 most appropriate algae species for enhanced nutrient removal from wastewater in temperate
328 climates remains unclear and highlights that understanding the overall impact of the use of
329 algae cannot be determined without knowledge of the species involved.

330

331 CONCLUSIONS

332 The adoption of low energy harvest and algal biomass pre-treatment has been shown to have
333 a significant impact on the overall suitability of using algae for nutrient removal in
334 wastewater treatment. The BDAF process reduced the overall energy requirements between
335 30 and 40% depending on the plant size. Thermal hydrolysis pre-treatment allowed a
336 complete utilisation of the included *S. obliquus* cells under mesophilic temperatures
337 maximising the potential energy gain from inclusion of the biomass. The combination of the
338 two technologies demonstrated the possibility of achieving high-energy efficiency (76%) and
339 a more sustainable WWTP. Adoption of the approach needs knowledge of the specific
340 species involved in the removal process as different strains of algae require different pre-
341 treatment conditions and will be able to release different amount of energy and this remains a
342 key research challenge going forward.

343 *Acknowledgment*

344 The authors would like to thanks the EU Framework 7 project Advance Technologies for
345 Water Resources and Management (ATWARM), Marie Curie Initial Training Network, No.
346 238273 as well as the Engineering and Physical Sciences Research Council (EPSRC),
347 Anglian Water, Severn Trent Water and Scottish Water for the financial and intellectual
348 support.

349

350 REFERENCES

- 351 [1] Martínez, M.E., Sánchez, S., Jiménez, J.M., El Yousfi, E., Munoz, L.: Nitrogen and
352 phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*.
353 Bioresource Technol. 73, 263-272 (2000)
- 354 [2] Xin, L., Hong-Ying, H., Jia, Y.: Lipid accumulation and nutrient removal properties of a
355 newly isolated freshwater microalga, *Scenedesmus sp.* LX1, growing in secondary
356 effluent. New Biotechnology. 27, 59-63 (2010)
- 357 [3] Ruiz-Marin, A., Mendoza-Espinosa, L.G., Stephenson, T.: Growth and nutrient removal
358 in free and immobilized green algae in batch and semi-continuous cultures treating real
359 wastewater. Bioresource Technol. 101, 58-64 (2010)
- 360 [4] Christenson, L., Sims, R.: Production and harvesting of microalgae for wastewater
361 treatment, biofuels, and bioproducts. Biotechnol. Adv. 29, 686-702 (2011)
- 362 [5] Demirbas, A.: Use of algae as biofuel sources. Energy Convers. Manage. 51, 2738-2749
363 (2010)
- 364 [6] Pittman, J.K., Dean, A.P., Osundeko, O.: The potential of sustainable algal biofuel
365 production using wastewater resources. Bioresource Technol. 102, 17-25 (2011)
- 366 [7] Min, M., Wang, L., Li, Y., Mohr, M.J., Hu, B., Zhou, W., Chen, P., Ruan, R.: Cultivating
367 *Chlorella sp.* in pilot-scale photobioreactor using centrate wastewater for microalgae
368 biomass production and wastewater nutrient removal. Appl. Biochem. Biotechnol. 165,
369 123-137 (2011)
- 370 [8] Tredici, M.R.: Mass production of microalgae: photobioreactors. In Richmond, A. (ed):
371 Handbook of microalgal culture: biotechnology and applied phycology. Blackwell
372 Science. 178-214 (2007)
- 373 [9] Cromar, N.J., Fallowfield H.J.: Effect of nutrient loading and retention time on
374 performance of high rate algal ponds. J. Appl. Phycology 9, 301-309 (1997)
- 375 [10] Molina Grima, E., Belarbi, E.H., Ación Fernández, F.G., Robles Medina, A., Chisti, Y.:
376 Recovery of microalgal biomass and metabolites: process option and economics.
377 Biotechnol. Adv. 20, 491-515 (2003)
- 378 [11] Salim, S., Vermuë, M.H., Wijffels, R.H.: Ratio between autoflocculating and target
379 microalgae affects the energy-efficient harvesting by bio-flocculation. Bioresource
380 Technol. 118, 49-55 (2012)
- 381 [12] Beach, E.S., Eckelman, M.J., Cui, Z., Brentner, L., Zimmerman, J.B.: Preferential
382 technological and life cycle environmental performance of chitosan flocculation for

- 383 harvesting of the green algae *Neochloris oleoabundans*. *Bioresource Technol.* 121, 445-
384 449 (2012)
- 385 [13] Edzwald, J.K.: Algae, bubbles, coagulants, and dissolved air flotation. *Wat. Sci. Tech.*
386 27, 67-81 (1993)
- 387 [14] Jarvis, P., Buckingham, P., Holden, B., Jefferson, B.: Low energy ballasted flotation.
388 *Water Res.* 43, 3427-3434 (2009)
- 389 [15] Mussnug, J.H., Klassen, V., Schlüter, A., Kruse, O.: Microalgae as substrates for
390 fermentative biogas production in a combined biorefinery concept. *J. Biotechnol.* 150,
391 51-56 (2010)
- 392 [16] Heaven, S., Milledge, J., Zhang, Y.: Comments on Anaerobic digestion of microalgae as
393 a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances* 29,
394 164 -167 (2011)
- 395 [17] Golueke, C.G., Oswald, W.J., Gotaas, H.B: Anaerobic digestion of algae. *Appl.*
396 *Biotechnol.* 5, 47-55 (1957)
- 397 [18] Zamalloa, C., Boon, N., Verstraete, W.: Anaerobic digestibility of *Scenedesmus obliquus*
398 and *Phaedactylum tricornutum* under mesophilic and thermophilic conditions. *Apply*
399 *Energy* 92, 733-738 (2012)
- 400 [19] Yen, H.W., Brune, D.E.: Anaerobic co-digestion of algal sludge and waste paper to
401 produce methane. *Bioresource Technol.* 98, 130-134 (2007)
- 402 [20] Gonzalez-Fernandez, C., Sialve, B., Bernet, N., Steyer, J.P.: Impact of microalgae
403 characteristics on their conversion to biofuel. Part II Focus on biomethane production.
404 *Biofuels Bioprod. Bioref.* 6, 205-218 (2012)
- 405 [21] González-Fernández, C., Sialve, B., Bernet, N., Steyer, J.P.: Thermal pretreatment to
406 improve methane production of *Scenedesmus* biomass. *Biomass Bioenerg.* 40, 105-111
407 (2012)
- 408 [22] Abo-Shady. A.M., Mohamed, Y.A., Lasheen, T.: Chemical composition of the cell wall
409 in some green algae species. *Biol. Plantarum.* 35, 629-632 (1993)
- 410 [23] Ehime, E.A., Holm-Nielsen, J.B., Poulsen, M., Boelsmand, J.E.: Influence of different
411 pre-treatment routes on the anaerobic digestion of a filamentous algae. *Renew. Energ.* 50,
412 476-480 (2013)
- 413 [24] Alzate, M.E., Munoz, R., Rogalla, F., Fdz-Polanco, F., Pérez-Elvira S.I.: Biochemical
414 methane potential of microalgae: influence of substrate to inoculum ratio, biomass
415 concentration and pretreatment. *Bioresource Technol.* 123, 488-494 (2012)

- 416 [25] Shi, C.Y.: Mass flow and energy efficiency of municipal wastewater treatment plants.
417 IWA Publishing, London (2011)
- 418 [26] Dutch Foundation for Applied Water Research (STOWA): Cambi process sheets. Web.
419 www.stowa-selectedtechnologies.nl/Sheets/Cambi.Process.html (2006). Accessed 18
420 May 2012
- 421 [27] Henderson, R.K., Parsons, S.A., Jefferson, B.: The potential for using bubble
422 modification chemicals in dissolved air flotation for algae removal. *Sep. Sci. Technol.*
423 44, 1923-1940 (2009)
- 424 [28] Greenberg, A.E., Clesceri, L.S., Eaton, A.D.: Standard methods for the examination of
425 water and wastewater. APHA (1992)
- 426 [29] Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J.,
427 Kalyuzhnyi, S., Jenicek, P., van Lier J.B.: Defining the biomethane potential (BMP) of
428 solid organic waste and energy crops: a proposed protocol for batch assays. *Water Sci.*
429 *Technol.* 59, 927-934 (2009)
- 430 [30] Zamalloa, C., Vulsteke, E., Albrecht, J., Verstraete W.: The techno-economic potential
431 of renewable energy through the anaerobic digestion of microalgae. *Bioresource Technol.*
432 102, 1149-1158 (2011)
- 433 [31] Henderson, R.K., Parsons, S.A., Jefferson, B.: The impact of differing cell and algogenic
434 organic matter (AOM) characteristics on the coagulation and flotation of algae. *Water*
435 *Res.* 44, 3617-3624 (2010)
- 436 [32] Granados, M.R., Acien, F.G., Gomez, C., Fernandez-Sevilla, J.M., Molina Grima, E.:
437 Evaluation of flocculants for the recovery of freshwater microalgae. *Bioresource*
438 *Technol.* 118, 102-110 (2012)
- 439 [33] Ras, M., Laurent, L., Sialve, B., Bernet, N., Steyer, J.P.: Experimental study on a coupled
440 process of production and anaerobic digestion of *Chlorella vulgaris*. *Bioresource*
441 *Technol.* 102, 200-206 (2011)
- 442 [34] Valo, A., Carrère, H., Delgenès, J.P.: Thermal, chemical and thermo-chemical pre-
443 treatment of waste activated sludge for anaerobic digestion. *J. Chem. Technol. Biot.* 79,
444 1197-1203 (2004)
- 445 [35] Austin, D., Nivala, J.: Energy requirement for nitrification and biological nitrogen
446 removal in engineered wetlands. *Ecol. Eng.* 35, 184-192 (2009)
- 447 [36] Syrett, P.J., Thomas, E.M.: The assay of nitrate reductase in whole cells of *Chlorella*:
448 strain differences and the effect of cell walls. *New Phytol.* 72, (1307-1310)

- 449 [37] Henderson, R.K., Chips, M., Cornwell, N., Hitchins, P., Holden, B., Hurley, S., Parsons,
450 S.A., Wetherill, A., Jefferson, B.: Experiences of algae in UK waters: a treatment
451 perspective. *Water and Environment Journal*. 22, 184-188 (2008)
- 452 [38] Henderson, R.K., Parsons, S.A., Jefferson, B.: The impact of variable algal functionality
453 on treatment. *Water Res.* 42, 1827-1845 (2008)
- 454 [39] Strum, B.S.M., Lamer, S.L.: An energy evaluation of coupling nutrient removal from
455 wastewater with algal biomass production. *Appl. Energ.* 88, 3499-3506 (2011)
- 456 [40] Sialve, B., Bernet, N., Bernard, O.: Anaerobic digestion of microalgae as a necessary
457 step to make microalgal biodiesel sustainable. *Biotechnol. Adv.* 27, 409-416 (2009)