

Accepted Author Manuscript.

Treatment of food waste digestate using microalgae-based systems with low-intensity Light Emitting Diodes (LEDs)

Andrés Felipe Torres Franco^a; Scarlet da Encarnação Araújo^b; Fabiana Passos^a, Carlos Augusto de Lemos Chernicharo^a, César Rossas Mota Filho^{ac}, Cleber Cunha Figueredo^b.

^a Department of Sanitation and Environmental Engineering, Federal University of Minas Gerais (DESA-UFMG), Brazil

^b Institute of biologic Sciences, Federal University of Minas Gerais (DESA-UFMG), Brazil

^c corresponding autor, cesar@desa.ufmg.br

Abstract

Anaerobic digestion of food wastes coupled with digestate post-treatment using microalgae-based systems could recover large amounts of energy and nutrients worldwide. However microalgae inhibition by high ammonia concentrations and low light transmittances affecting photosynthesis should be overcome to develop full scale implementations. This study evaluated the potential of microalgae-based reactors supplied with red LEDs at low intensity (660 nm and $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to treat food waste digestate. LED reactors were compared with control reactors exposed to natural solar radiation. From a range of species in the inoculum, *Chlorella vulgaris* showed high adaptation to both lighting regimes and digestate environmental conditions, characterized by a C:N:P ratio of 74:74:1. Removal efficiencies for control and LED reactors were 84.0% and 95.8 % for soluble COD and 89.4% and 53.0 % for ammonia, respectively. Approximately 50% of ammonia in control reactor and 15% in

Accepted Author Manuscript.

21 LED reactor was lost mainly through volatilization, whereas 17% and 36% of ammonia was
22 transformed in organic nitrogen in control and LED reactors, respectively. Low-intensity
23 LEDs maintained microalgae growth in levels similar to solar radiation and supported
24 efficient digestate treatment, showing a potential for further application in optimization of
25 full scale reactors at a relatively low energy cost.

26 **Keywords**

27 *Chlorella vulgaris*; food waste digestate; microalgal-based treatment; light-emitting diodes

28

29 **Introduction**

30 Interest in anaerobic processes to treat food waste has grown intensely in the last decades due
31 to its multiple advantages, including the possibility to recover energy. However, the liquid
32 effluent from this process (i.e. digestate) usually contains high concentrations of organic
33 matter, ammonia, phosphorus (Möller & Müller, 2012) and, sometimes, pathogens (Sheets
34 *et al.*, 2015). Nutrients in food waste digestate could be recovered and applied in agriculture.
35 However, direct application of digestate in agriculture is not recommended, as its carbon to
36 nitrogen ratio could be not adequate for plant growth, which could also cause environmental
37 impacts by e.g ammonia volatilization.

38 Microalgae-based technologies, such as stabilization ponds, high-rate ponds and
39 photobioreactors, have been applied as a promising alternative to recover nitrogen and
40 phosphorus from food waste digestates (Mayers *et al.*, 2017). However, there are many
41 aspects of these processes that require further research and improvement. For instance,

Accepted Author Manuscript.

42 residual solids may limit light penetration, high levels of NH₃ in the digestate may cause
43 inhibitory effects to microalgal growth and predators could significantly affect microalgae
44 productivity (Sheets *et al.*, 2015; Tricolici *et al.*, 2014). Dilution has been used as a strategy
45 to avoid ammonia inhibition and solids interference in light penetration, although it may not
46 be a cost-effective alternative for full-scale implementation since it may demand high
47 amounts of water, making that option unfeasible or unsustainable. High organic carbon and
48 ammonia removals have been achieved at relatively short hydraulic retention times (HRT)
49 with the photo-activated sludge process, where consortia of microalgae and nitrifying
50 bacteria removed ammonium efficiently (van der Steen *et al.*, 2015). Photosynthesis in
51 photobioreactors may ease oxygen limitations for bacterial growth, while artificial lighting
52 can provide adequate photosynthetic active radiation (PAR) for microalgae growth. The use
53 of light emitting diodes (LED) as a light source for algal cultures have gained more attention
54 in recent years since they can produce higher levels of radiation with relatively low energy
55 demand, heating, carbon and area footprints (Mohamed *et al.*, 2014), so their use may be a
56 strategy to overcome shadowing effects of solids and optimize biomass production. Previous
57 studies suggest that red light (particularly at 660 nm) at moderate to high light intensities
58 (i.e., 50- 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was optimal for microalgae growth in wastewaters and
59 digestates (Zhao *et al.*, 2013; Mohammed *et al.*, 2014). However, those light intensities may
60 still represent high energy consumption ($0.54 \text{ kWh}\cdot\text{kgCOD}_{\text{removed}}^{-1}$) and even exceed
61 photoinhibition levels reported for strains like *C. vulgaris*. under artificial lighting (Maxwell
62 *et al.*, 1995).

Accepted Author Manuscript.

63 In order to enhance photobioreactors for microalgae based treatment of concentrated
64 digestates and wastewaters, LED lighting can be used to increase photic depth in hybrid
65 ponds and tanks, reducing area requirements but still taking advantage of natural solar
66 radiation, particularly in tropical climates. However, it is required to determine the minimum
67 levels of PAR for microalgae photosynthesis and global treatment efficiencies. Thus, the
68 current work intended to explore the potential of low intensity LEDs to support phototrophic
69 activity in a microalgae-based system to treat food waste digestate under the hypothesis that
70 a minimum level of $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ can sustain photosynthesis and become a minimum value
71 of reference for artificial lighting in further full scale implementations.

72 **Material and Methods**

73 *Experimental set-up*

74 Laboratory-scale photobioreactors were operated in batch mode treating food waste digestate
75 from a UASB reactor (Figure 1). The reactors consisted of six transparent acrylic tubes with
76 a working volume of 800 mL, which were arranged vertically outdoor at the Engineering
77 School of the Federal University of Minas Gerais (Belo Horizonte, Brazil- $19^{\circ}52'11.04''\text{S}$,
78 $43^{\circ}57'42.92''\text{W}$). Mixing was achieved by motors operating at 6 rpm, installed at the top of
79 each reactor and welded to a vertical shaft with mechanical paddles. Red LED lights in
80 dimmerizable strips (wavelength 660 nm, $\sim 25\text{ W}$) were installed at the wall of the reactors
81 (LED reactor), which were then covered with aluminum foil in order to supply a PAR of 15
82 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Control reactors were exposed to natural solar radiation with a daily mean PAR
83 of $580 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Photoperiod for LED reactors was fixed in 12 hours, which was

Accepted Author Manuscript.

84 approximately the same time between dawn and sunset. Both LED and control reactors were
85 built and operated in triplicates.

86 *Food waste digestate, microalgae and Activated Sludge Inoculum*

87 Food waste digestate was obtained from a treatment system composed by an anaerobic
88 completely stirred tank reactor, followed by a UASB reactor operated in series and fed with
89 500 kg of food waste per week obtained from of the university canteens (UFMG, Belo
90 Horizonte, Brazil).

91

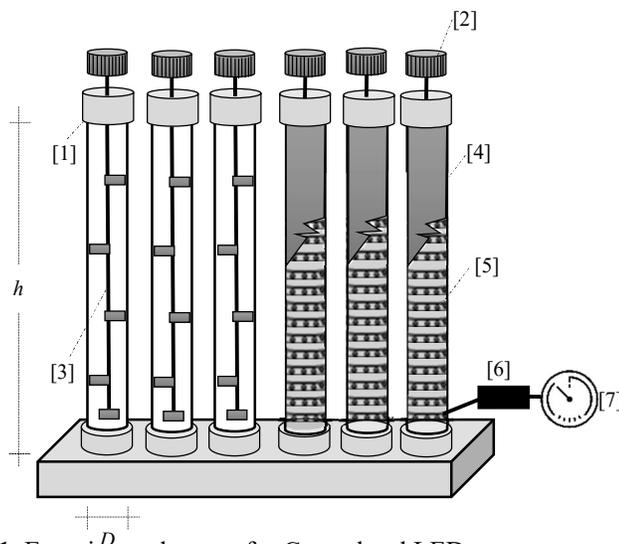
92

93

94

95

96



97

98

99

Figure 1. Experimental set-up for Control and LED reactors composed of [1] transparent acrylic tube $D=4$ cm; $h=54$ cm, [2] Motors at 6 rpm [3] Vertical shaft and paddles (2 cmx1cm); [4] Aluminum foil; [5] Led strips (660 nm), ~ 5 w/m, 110 V. [6] Wireless dimmers [7] Automatic timer

100

101 Microalgae inoculum was obtained from a maturation pond treating municipal wastewater
102 with high relative abundance of *Euglena* sp., *Scenedesmus* sp. and *Chlorella vulgaris*. To
103 increase the microalgae diversity in the initial inoculum, natural samples obtained from an

Accepted Author Manuscript.

104 urban reservoir in Belo Horizonte were concentrated with a phytoplankton net and added at
105 a 1:1 ratio. For inoculation, 500 mL of the microalgae previously collected were suspended
106 with 2 L of food waste digestate and 2.5 L of distilled water to avoid ammonia toxicity, which
107 resulted in total ammonia nitrogen (TAN) and total suspended solids (TSS) concentrations
108 of $100 \text{ mg}\cdot\text{L}^{-1}$ and $500 \text{ mg}\cdot\text{L}^{-1}$, respectively. Each reactor was inoculated with 500 mL of this
109 mixture. After an acclimation period of three days (Period I), total suspended solids (TSS)
110 were measured and activated sludge from a municipal wastewater treatment plant (Belo
111 Horizonte, Brazil) was added after 1 h settling at a ratio of 5:1 (microalgae:activated sludge).
112 Working volume of each reactor was completed to 800 mL with a new pulse of food waste
113 digestate and maintained in operation during 15 d (Period II).

114 COD, TAN and $\text{PO}_4\text{-P}$ were determined on filtered samples every 48 h. Operational
115 parameters including, pH, dissolved oxygen (DO), temperature (T) and total suspended solids
116 (TSS) were measured with the same frequency, and at 12noon. Quantification of total
117 Kjeldahl nitrogen (TKN), nitrate ($\text{NO}_3^- \text{-N}$), coliforms and *Escherichia coli*, as well as
118 microalgae (by microscopy) was performed in the third day, immediately after the addition
119 of activated sludge and digestate, and in the last day of the experiment. Flow cytometry
120 analyses were performed for samples taken from inoculum and reactors at the end of the
121 operational period to establish the ratio of cells fluorescing to red light (670 LP Filter).

122 ***Experimental analyses***

123 Food waste digestate was characterized by measuring Total COD, soluble COD (S-COD),
124 total ammonia nitrogen (TAN), organic nitrogen (Org-N), $\text{NO}_3\text{-N}$, soluble reactive

Accepted Author Manuscript.

125 phosphorus ($\text{PO}_4\text{-P}$), total phosphorus (Total-P), pH, total alkalinity, TSS and volatile
126 suspended solids (VSS) according to *Standard Methods of Water Examination* (Rice *et al.*,
127 2012).

128 DO and temperature were measured directly in the reactors using a sensor (Hach® HQ30d)
129 connected to a probe (Hach® LDO101). pH was quantified using a benchtop pH-meter
130 (Denver Instruments® UB-5). Concentrations of $\text{NO}_3^- \text{-N}$ were measured by NitraVer® 5
131 Nitrate Reagent Powder Pillows (HM 8039). Total coliforms and *E. coli* were measured using
132 the chromogenic substrate (Colilert-18/Quanti-Tray) technique. Microalgae populations
133 were quantified by Utermöhl technique using an inverted microscope (Zeiss, Primovert -
134 Germany). Chlorophyll-a (*Chl-a*) content was assessed by ethanol extraction and quantified
135 by measuring absorbances at 645 and 663 nm with a spectrophotometer and using Becker's
136 equation. For flow cytometry, samples were fixed in Paraformaldehyde and were evaluated
137 using a FACSCanto™ II Cytometer. Side scatter and the 488/530/30 laser and detectors were
138 used. The fraction of cells fluorescence under red light was measured with a 670 LP Filter.
139 Milli-Q water, saline solution and activated sludge were used as negative controls for flow
140 cytometry.

141 Light intensity in the reactors was measured using a radiometer (Li-cor Li-189, USA).
142 System's performance was assessed in terms of *Chl-a* productivity and COD, TAN and $\text{PO}_4\text{-P}$
143 removal. *Chl-a* productivity was estimated according to Eq. 1 from *Chl-a* triplicates
144 obtained for the first and last days of the operational time $[\text{Chl}]_{o/f}$, considering A as the
145 illuminated area ($A=\pi Dh$) and t as the number of days of the operational period and V_i and
146 V_f as initial and final volumes in each reactor.

Accepted Author Manuscript.

$$147 \quad \text{Chl} - a \text{ productivity} = \frac{V_f[\text{Chl}]_f - V_i[\text{Chl}]_i}{A \cdot t} \quad (\text{Eq. 1})$$

148 Removal efficiencies were determined for S-COD, TAN and PO₄-P.

149 *Statistical analysis*

150 Statistical analysis included basic descriptive statistics and a Repeated-Measures ANOVA
151 followed by a Bonferroni test performed in SPSS® for control and LED reactors, as between-
152 subject factors and sampling time as within-subject factor for a significance level at $p < 0.05$.
153 Values of F indicating differences are reported for the effects of time within reactor (F
154 calculated for the effect of the interaction *time-reactor*) and between reactors (overall
155 comparison). Normality was tested with a Shapiro-Wilk test. Data which did not follow
156 normality were submitted to a $\log(1+x)$ or a $-1/\ln X$ transformation in order to perform the
157 ANOVA. Homocedasticity was checked with Mauchly's Test of Sphericity and Greenhouse-
158 Geiser df corrections was used for autocorrelation when necessary.

159 **Results and Discussion**

160 *Food waste digestate characterization*

161 Food waste digestate characterization is summarized in Table 1. The digestate presented high
162 concentrations of organic matter, ammonia, phosphorus and solids. Relatively high pH and
163 total alkalinity are common in food wastes, which are caused by ammonia bicarbonate
164 buffers, CO₂ species concentrations of alkali compounds and bicarbonate and carbonate ions
165 formed by successive dissociation reactions of carbonic acid produced during anaerobic
166 digestion (Kang *et al.*, 2017). Org-N was very low since the digestate was obtained from a

Accepted Author Manuscript.

167 food waste methanization reactor coupled to a UASB reactor and thus, all organic nitrogen
168 was previously ammonified.

169 Nutrients concentrations were high enough to support microalgae growth in control and LED
170 reactors. Total Alkalinity in food waste digestate presented high concentrations and secured
171 inorganic carbon for autotrophic growth. An experimental C:N ratio based on S-COD of 1:1
172 and a N:P ratio of 73:1 suggest that carbon and phosphorus could have limited the total
173 assimilation of TAN by heterotrophic biomass, which were possible affected since C:N ratio
174 was relatively low when compared with optimum ratios reported for *Chlorella* sp. cultivated
175 in wastewater with artificial light supplying (C:N ratio of 5:1 to 10:1) (Yan *et al.*, 2013) and
176 optimum N:P ratio for reaching a maximum biomass production of *Chlorella* and nutrients
177 removal (~10:1) (Choi & Lee, 2015).

178

179 **Table 1.** Physicochemical characterization of the initial condition of the food waste digestate
180 used in the control and in the treatments using LED reactors

Parameter	Value (mean ± std. dev.)	Units
Total COD	1856.7±118.1	mg·L ⁻¹
Filtered COD (S-COD)	808.3±125.2	mg·L ⁻¹
TAN	802.1±20.3	mg·L ⁻¹
TKN	805.1±18.2	mg·L ⁻¹
NO ₃ -N	12.5±6.46	mg·L ⁻¹
PO ₄ -P	4.9 ±7.4	mg·L ⁻¹

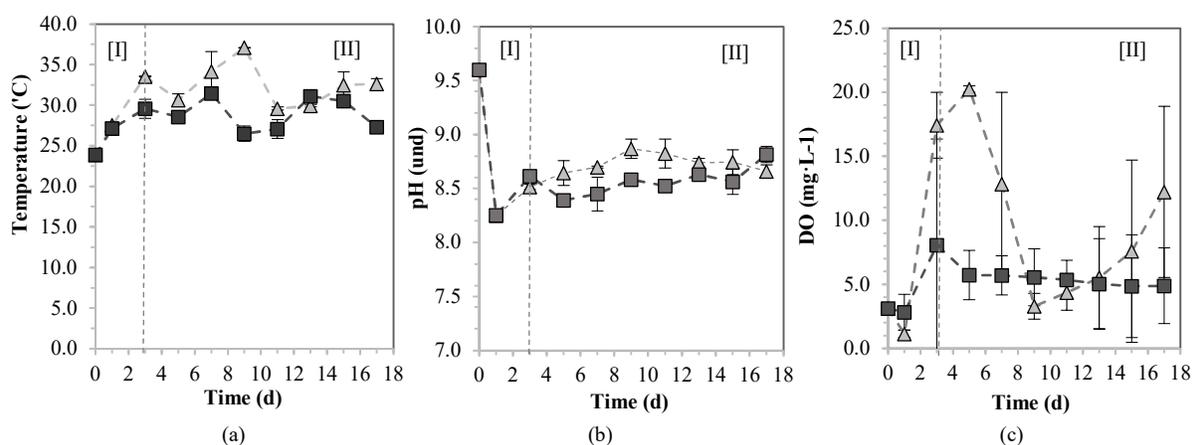
Accepted Author Manuscript.

Total-P	10.9±2.5	mg·L ⁻¹
pH	7.82	Und
Total Alkalinity	4116.8	mgCaCO ₃ ·L ⁻¹
TSS	1225±125	mg·L ⁻¹
VSS	1045±55	mg·L ⁻¹

181

182 Operational parameters

183 Temperature, pH and DO are shown in Figure 2. The control reactors presented a mean
 184 temperature of 31.3±3.2 °C, whereas the LED reactors temperature was 28.6±2.2 °C. No
 185 significant differences were found between the reactor's temperatures ($F=6.713$, $p>0.05$)
 186 nor within the reactors, along the operational time ($F=0.253$, $p>0.05$). pH ranged between
 187 8.24 and 9.60 for control reactors, and between 8.22 and 9.60 for the reactors with LED.
 188 Mean DO values of 9.1±7.8 and 9.0±6.4 mg DO/L were measured for the control and LED
 189 reactors, respectively. No significant differences were detected for pH and DO within (pH:
 190 $F=2.709$ $p>0.05$ and DO: $F=23.395$ $p>0.05$) or between reactors (pH: $F=4.199$ $p>0.05$ and
 191 DO: $F=4.056$ $p>0.05$)



192
193

Accepted Author Manuscript.

194 **Figure 2.** Temporal series of (a) Temperature; (b) pH and (c) DO for [I] Acclimation and [II] batch period,
195 for [▲] Control and [■] LED reactors.

196 While temperature was an independent and not controlled variable, pH and OD were
197 determined by biological processes. Constant pH values were accompanied by alkalinity
198 decreases detected in both reactors. Total alkalinity consumption was 885.4 ± 7.5 mg CaCO₃
199 in the control reactors and 440.9 ± 113.6 mg CaCO₃ in the LED reactors. These decreases
200 were attributed to microalgae consumption of dissolved CO₂ during photosynthesis (Park *et*
201 *al.*, 2011), as no precipitate formation or other CO₂ loss pathways occurred in the reactors.
202 In addition, microalgae photosynthesis was probably the major cause of the tendencies
203 observed for DO, while showing that measured concentrations were enough for holding
204 heterotrophic activity. Oxygen consumption was observed after the addition of activated
205 sludge containing bacterial populations, resulting in a slight decrease in DO concentrations.
206 The concentrations of oxygen in the control reactors showed a wider variation when
207 compared to the LED reactors. Control reactors reached maximum DO concentrations
208 around 20 mg/L due oversaturation produced by intense photosynthetic activity during
209 hours of maximum sunlight, resulting in a high standard deviation of DO values but no
210 significant differences when compared to LED reactors, according to the results of repeated
211 measures ANOVA. Conversely, LED reactors exhibited shorter variations since lighting
212 intensity and consequently the photosynthetic rates were always relatively constant.

213 **Microalgae production**

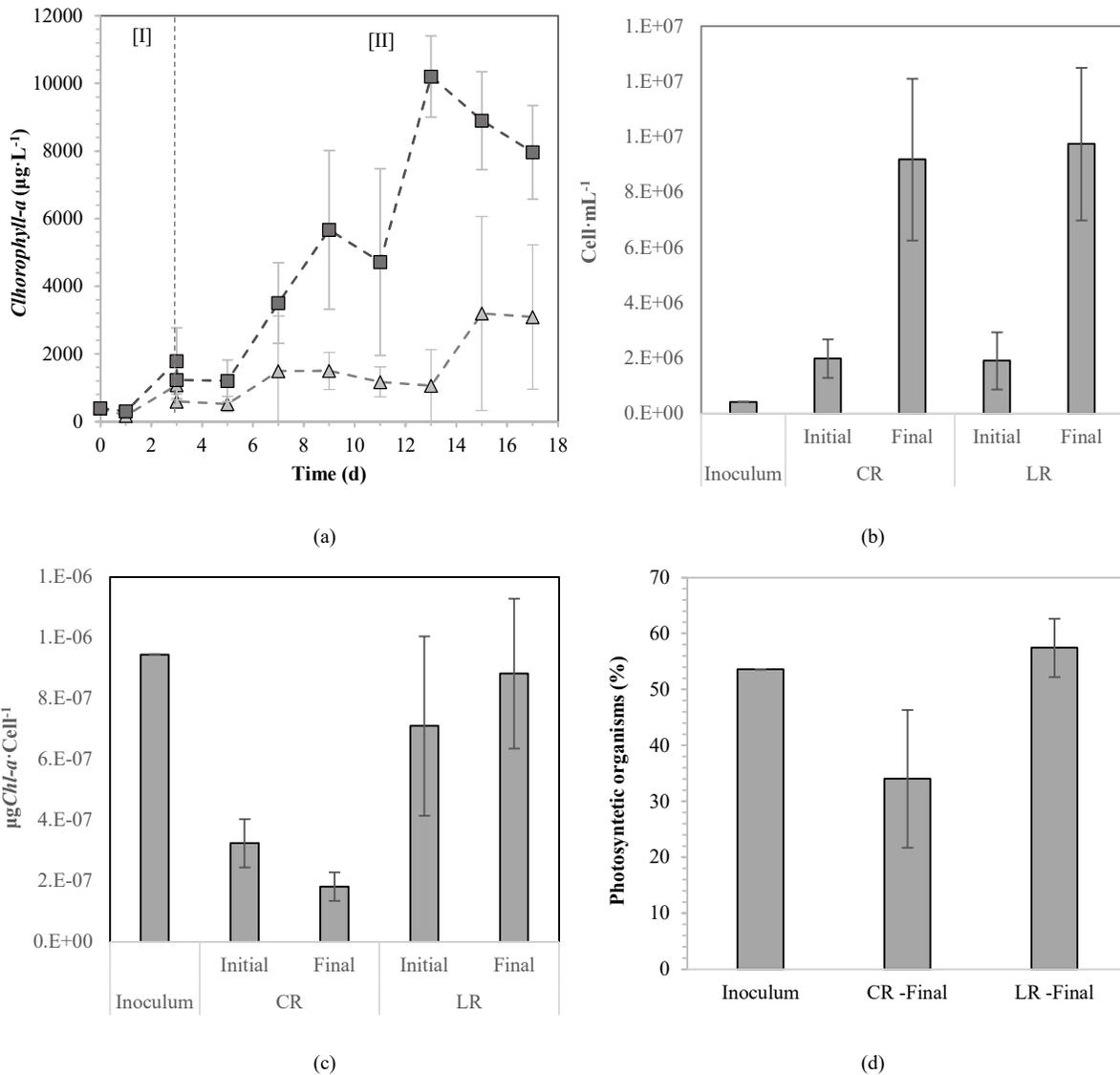
214 Chlorophyll-a (*Chl-a*) concentrations were evidence that microalgae used the digestate as
215 source of nutrients in both lighting conditions (Figure 3a). However, significant differences

Accepted Author Manuscript.

216 were detected within ($F=18,136$, $p<0.05$) and between reactors ($F=52.037$, $p<0.05$),
217 whereby light conditions using LED were considerably better for microalgal biomass
218 production in respect to solar lighting provided in control reactors. *Chl*-productivity in
219 control and LED reactors at the end of the operational period were of 2.9 ± 2.1 and 31.3 ± 6.4
220 $\text{mg Chl-}a\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, respectively.

221 Chlorophyll increased in relation to *Chlorella vulgaris* growth (Figure 3b), which was the
222 only species present in both reactors from the beginning of the acclimation (Period I) to the
223 end of the operational period (Period II). The adaptation of *C. vulgaris* to grow on digestate
224 and both lighting conditions was confirmed by the increases in cell counting along the
225 acclimation and operational periods. Regarding microalgae growth, average net value for
226 LED reactors was slightly higher than for control, *i.e.* $7.83\cdot 10^6$ and $7.20\cdot 10^6$ $\text{cell}\cdot\text{mL}^{-1}$,
227 respectively and more *Chl-a* content was measured in that treatment (Figure 3c). These
228 results matched with fluorescence under red light (670 LP filter) in flow cytometry,
229 indicating that the percentage of photosynthetic organisms (Figure 3d) increased in the
230 LED reactors (*i.e.* $57.4\pm 5.2\%$) and decreased in the control (*i.e.* $34.2\pm 12.3\%$). This was
231 possibly a consequence of differences in bacteria and microalgae cell content of
232 Chlorophyll. Additionally, heterotrophic bacteria (non-fluorescing cells) seemed to increase
233 their populations in the control, while remaining relatively constant when using LED.

Accepted Author Manuscript.



234

235

236

237

238

239

240

241

242

243

Figure 3. Temporal series of (a) Chlorophyll-a for [I] Acclimation and [II] operational period, for [▲]

Control and [■] LED reactors, (b) *Chlorella vulgaris* counting of inoculum and initial and final day of operational period (Period II) for Control Reactors (CR) and LED reactor (LR), (c) *Chl-a* content per cell at the initial and final day of operational period and (d) Percentage of photosynthetic organism (Fluorescence to red light with a 670 LP Filter at FACSCanto™ II Cytometer)

Accepted Author Manuscript.

244 More *Chl-a* content per cell the LED reactors suggests that algae responded to the light
245 condition by altering their photosynthetic pigment content (Wu, 2016). It is also possible
246 that LED treatment produced changes in cell sizes since larger cells would have greater
247 amount of chlorophyll for same net biomass quantities, but this was not verified as no
248 measures of cell sizes were made. Cell counting and *Chl-a* content were similar to previous
249 values found in outdoor ponds treating wastewater with ammonia concentrations in the
250 range of 800-1600 mg/L and pH 8. However, *Chl-a* content in LED reactors in our study
251 was higher than those previously reported (Ayre *et al.*, 2017).

252 LED light at low intensities also seemed to promote a more energy-efficient photosynthesis
253 compared to high PAR intensity sunlight in control reactors, which may have been closed
254 to photoinhibition levels. Probably, the reason for this inefficiency is related to a higher
255 photon flux density, in which the rate of photon absorption by the antenna chlorophylls
256 exceeds the rate at which photosynthesis occur at maximum, wasting up to 50– 80% of
257 absorbed photons (Carvalho *et al.*, 2011). Additionally, since each strain and culture may
258 experience photoinhibition at different intensities and culture conditions (Sorokin &
259 Krauss, 1958), our results suggest that adaptation of *C. vulgaris* to food waste digestate at
260 outdoor Brazilian temperatures seemed to perform better at low artificial lighting intensities
261 at 660 nm than at high intensities of natural sunlight. Furthermore, low intensities may
262 represent a more cost-efficient solution for wastewater treatment in photobioreactors in
263 respect to higher artificial lighting intensities previously reported (Yan *et al.*, 2013; Zhao *et*
264 *al.*, 2013; Mohammed *et al.*, 2014).

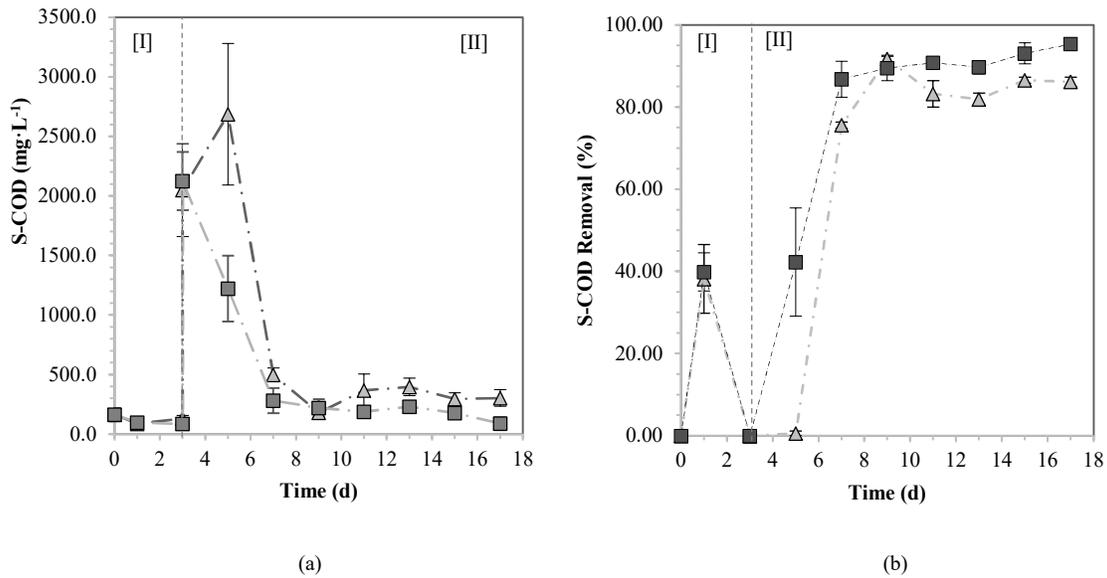
Accepted Author Manuscript.

265 In respect to species diversity, food waste digestate showed an inhibitory effect, since
266 original inoculum had a higher number of species, composed of *Scenedesmus* spp., *Euglena*
267 spp., *Chlorella Dynobryon* sp., *Monactinus simplex*, *Botryococcus braunii*, *Pandorina* sp.,
268 *Planktolyngbya limnetica*, *Aulacoseira granulata*, *Peridinium* sp, *Desmodesmus* sp,
269 *Trachelomonas volvocina* and *Microcystis aeruginosa*. However, the diversity was quickly
270 lost during the first days of the experiment. This was probably related to high ammonia
271 concentrations in the digestate. In fact, ammonia toxicity is a main constrain in microalgae-
272 based for highly concentrated wastewater treatments, such as observed for *Scenedesmus*
273 *obliquus* produced with 534 mg NH₄Cl·L⁻¹ at pH 8.4 (Azov and Goldman, 1981) or
274 *Neochloris oleoabundans* and *Dunaliella tertiolecta*, inhibited at levels of 2.3 and 3.3 mg
275 NH₃·L⁻¹ at pH 8.0 (Gutierrez *et al.*, 2016). However, some species may survive under high
276 ammonia concentrations, such as *Chlorella sorokiniana* and *Nannochloropsis oculata*
277 (Gutierrez *et al.* 2016), which could be a similar response observed for *Chlorella vulgaris*
278 in our experiments.

279 ***Digestate treatment performance***

280 ***S-COD Removal.*** S-COD concentrations significantly decreased along the
281 operational period within both reactors (Figure 4a) (F=81.68, p<0.05) with final
282 concentrations of 304.2±77.1 and 84.0±17.4 mg·L⁻¹, in control and LED reactors,
283 respectively (F=17.516, p<0.05). S-COD removal efficiencies increased within reactors
284 along the operational period (F=10.529 p<0.05) (Figure 4b) leading to final S-COD
285 removal efficiencies of 84.0±4.1 in control and significantly higher efficiencies of
286 95.8±0.92% in LED reactors (F=21.695, p<0.05).

Accepted Author Manuscript.



287
288
289 **Figure 4.** Temporal series for (a) Soluble COD (S-COD) and (b) S-COD removal efficiencies for [I]

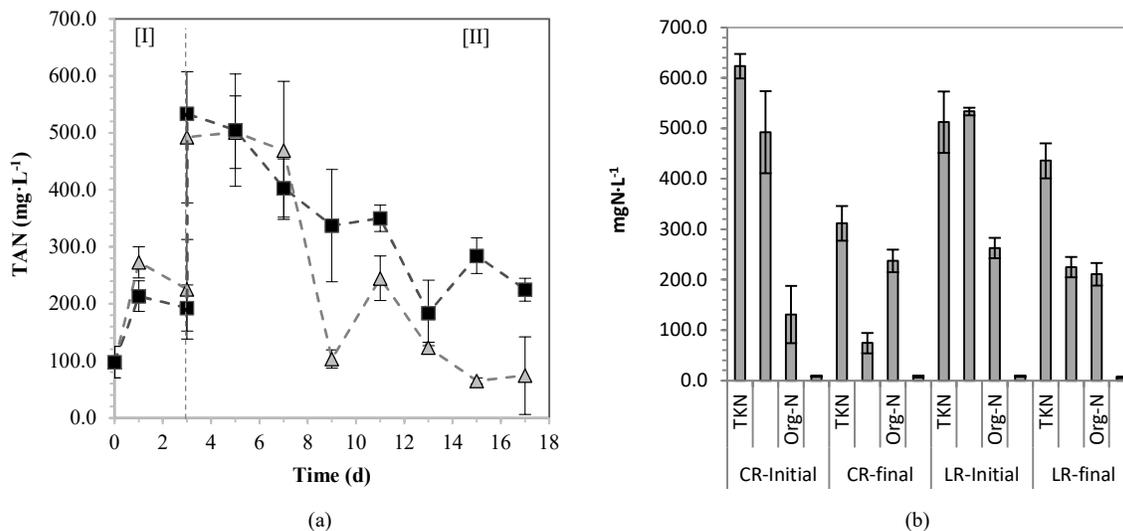
290 Acclimation and [II] operational period, for [▲] Control and [■] LED reactors

291
292 S-COD Removal efficiencies above 80% were obtained in both LED and control reactors
293 after 6 to 8 days of experiment. Higher efficiencies of S-COD removal using LED
294 suggested that low irradiances of $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ did not limit microalgae-based treatment
295 of food waste digestate and, conversely, exhibited a better performance compared to other
296 studies in which LED lighting at higher irradiances were (70-98%) (Yan *et al.*; 2013; Zhao
297 *et al.*, 2013; Mohammed *et al.*, 2014; Tricolici *et al.*, 2014). Lighting conditions in LED
298 reactors contributed to S-COD removal performance, since microalgae performed
299 photoaeration for heterotrophic activity without increasing pH to maximum values
300 unfavorable for activated sludge bacteria, while slightly higher maximum pH in control
301 reactors may have limited heterotrophic bacterial activity (Mohammed, 2014).

302 **Nitrogen transformations.** Figure 5(a) shows the temporal series for ammonia
303 nitrogen and (b) a nitrogen compounds balance for initial and final day of operational

Accepted Author Manuscript.

304 period. Total Ammonia Nitrogen (TAN) concentrations decreased over time within reactors
305 ($F=15.402$, $p<0.05$) and were significantly ($F=45.302$, $p<0.05$) lower in control reactors
306 (74.4 ± 48.1 mgTAN·L⁻¹) than in LED reactors (225.0 ± 20.3 mgTAN·L⁻¹), which removed
307 less ammonia ($53.0\pm 3.99\%$) than control reactors ($89.4\pm 6.07\%$).



308
309

310 **Figure 5.** (a) temporal series for ammonia nitrogen for [I] Acclimation and [II] operational period, for [▲]
311 Control and [■] LED reactors and (b) Nitrogen balance for initial and final day of the operational period [II]

312 The balance among different forms of nitrogen seems to be mainly influenced by ammonia
313 volatilization and biological assimilation. Approximately 17% and 36% of TAN was
314 transformed in Org-N in control and LED reactors, respectively, whereas approximately
315 50% of TAN in CR and 15% in LED reactor were lost from the system and unable to be
316 account in nitrogen balance. The increase in the Org-N fraction evidences that high
317 ammonia levels in FW digestate were not inhibitory for *C. vulgaris*, which was capable of
318 ammonia uptake for biomass production. Higher nitrogen losses in control reactors may be
319 attributed mainly to NH₃ volatilization caused by maximum pH values (>9.0) and
320 temperatures (~35 °C), which altered the NH₃/NH₄⁺-N equilibrium to more than 50% of

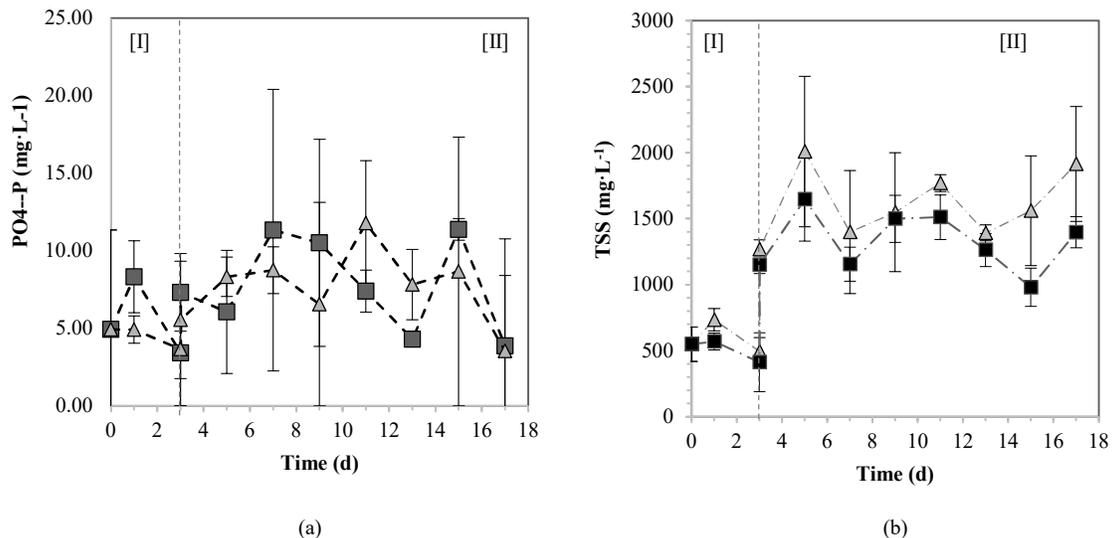
Accepted Author Manuscript.

321 ammonia being present as the neutral and volatile form (NH_3) and potentially volatilized
322 (Emerson *et al.*, 1975). Other mechanisms may include nitrate denitrification during
323 nighttime when DO concentrations were low ($< 1 \text{ mg/L}$) allowing the establishment of
324 anoxic conditions (Park & Craggs, 2011). However, nitrification was low in both
325 treatments since a short production of NO_3^- -N was observed, meaning that nitrifiers were
326 possibly inhibited by pH conditions above the optimum range (6.2 – 8.0) (Gujer, 2010).
327 TAN removal accounted for 83.7 ± 12.6 in control reactors and $61.2 \pm 9.1\%$ in LED reactors.
328 The efficiencies for nitrogen removal were relatively low when compared with other
329 studies using or not LED lighting (*e.g.* Tricolici *et al.*, 2014), but they are still results
330 valuable since initial concentrations at FWD were high and dilution was short ($\sim 30\%$).
331 From a sustainable reuse perspective, TAN levels in filtered effluent and Org-N in algal
332 biomass can be positive if soil application is planned as a reuse alternative for the system's
333 effluent and/or energetic exploitation of biomass.

334 ***Phosphorus and TSS.*** Statistical analyses of PO_4 -P concentrations (Figure 6a)
335 revealed no significant differences neither within reactors along time ($F=0.188$, $P>0.05$) nor
336 between reactors ($F=0.032$, $p>0.05$). PO_4 -P consumption may have been masked by re-
337 mineralization of Total-P from organic matter in digestate or even of the algal biomass,
338 especially in control reactors where more losses may have occurred due to temperature and
339 lighting variations. Anyway, removal efficiencies broadly coincide with other experiences
340 with *C. vulgaris*, reporting low values of about 20% for N:P ratios between 61-70 (Choi &
341 Lee, 2015) or not a clear tendency at all (Tricolici *et al.*, 2014). As mentioned for TAN,
342 phosphorus concentrations in effluent may give an agriculture value to the treated digestate,

Accepted Author Manuscript.

343 even though higher TAN removal efficiencies are required to obtain an effluent with an N:P
344 ratio of 1:5, which would have a more interesting ratio for *e.g.* beans fertirrigation.



345

346

347

Figure 6. Temporal series for (a) PO₄-P and (b) TSS for [I] Acclimation and [II] operational period, for [▲]

348

Control and [■] LED reactors

349

Figure 6b presents time variations for TSS concentrations in both reactors, which remained

350

constant along the operational period within ($F=3.041$, $p>0.05$) and between reactors

351

($F=1.476$, $p>0.05$). Considering the high concentration of TSS in FW digestate, solids were

352

not associated with algal/bacterial biomass, even considering that biomass is a fraction of

353

solids. No significant solids removal was expected in the MAB reactors, since the major

354

lost would be a consequence of sinking and microalgae can slowly settle so external

355

mechanism like centrifugation or flocculation are required (Park *et al.*, 2011).

356

Finally, both control and LED reactors inactivated Total Coliforms (TC) and *E. coli* at

357

similar magnitudes (Table 2). Even if final dilutions made for determinations could have

358

been smaller, at a dilution level of 10^2 no presence of Coliforms or *E. coli* was found by the

359

end of the operational period, so reactors effluent would accomplish the WHO's (2000)

Accepted Author Manuscript.

360 standard of $<10^3$ FC/100ml for reuse in agriculture. Mechanisms of TC and *E. coli*
361 inactivation in maturation ponds and algal systems have been reported as associated to high
362 penetration of UV radiation, elevated pH (especially $\text{pH}>10$), elevated DO concentration
363 (favoring photooxidation) (von Sperling, 2002). Since pH was always lower than 10 in both
364 control and LED reactor and no UV radiation was expected in the LED reactor, predominant
365 mechanism must have been an oxidative stress produced by momentane high DO
366 concentrations.

367 **Table 2.** Performance of Control and LED reactors for Total Coliforms and *E. Coli*
368 inactivation

Parameter	Total Coliforms		E. Coli	
	Control	LED	Control	LED
C_o	$2.8 \cdot 10^5 \pm 1.7 \cdot 10^5$	$4.6 \cdot 10^5 \pm 3.4 \cdot 10^5$	$1.5 \cdot 10^3 \pm 1.0 \cdot 10^3$	$8.8 \cdot 10^3 \pm 6.0 \cdot 10^3$
C_f		$<10^2$		
Removal (log-units)	5.3 ± 0.3	5.6 ± 0.3	2.9 ± 0.4	3.8 ± 0.3

369

370 *Sustainability of MAB technologies application to FWD*

371 Global results of this work provide a perspective for MAB reactors design and operation for
372 increasing sustainability in treatment of sewage containing high concentrations of organic
373 matter and nutrients, as those observed in FWD. It was evidenced that microalgal
374 productivity and economy of energy can be improved by combining solar and artificial
375 LED lighting. In this work, PAR intensities of $15 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ were obtained with dimmed
376 LED strips consuming ~ 12.5 W during HRT of 15 d and a photoperiod of 12 h, which was

Accepted Author Manuscript.

377 equivalent to an energy daily consumption of 0.15 kWh. The overall consumption was of
378 $\sim 0.48 \text{ kWh} \cdot \text{kg}_{\text{removed COD}}^{-1}$ or ($1875 \text{ kWh} \cdot \text{m}^{-3}$), which is considerably less than
379 consumptions of activated sludge and/or artificial lighting at higher LED irradiances (*e.g.*
380 Mohammed *et al.*, 2014).

381 Furthermore, the area required for application of MAB technologies could potentially be
382 reduced by increasing reactors depth using artificial LED lighting applied under the level of
383 the euphotic zone, which would remain illuminated by sunlight. Area requirement limit
384 current applications of microalgae based technologies in urban and peri-urban areas of
385 developed and developing countries, which are the typical localization of biggest FW
386 sources. Thus, reducing area requirements is a decisive factor to full scale implementations
387 of microalgae based technologies for sustainable treatment of food waste digestate. An
388 example could be as following: if a typical high rate algal pond (HRAP, $h=0.30 \text{ m}$) treating
389 1 m^3 per day of FW digestate may require an area of $\sim 33 \text{ m}^2$, an optimized HRAP of $h=1.2$
390 m could reduce that requirement to 8 m^2 ($\sim 75\%$) using LED lighting at low irradiance in a
391 deep of 0.90 m , provided by inner vertical panels with a PAR source producing around 400
392 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, which can easily secure minimum values around $15 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in an
393 horizontal distance of 15 cm (Mohammed *et al.*, 2013), overcoming lighting blocking and
394 scattering by solids. Energy consumption in such a reactor will be in a range of 1406 kWh .
395 Current (2017) electric energy price in Belo Horizonte is $\text{US}\$0.09/\text{KWh}$, so
396 $\text{US}\$253.125/\text{month}$ would be required per m^3 of FW treated. The cost per m^2 of area in
397 Belo Horizonte is about $\text{US}\$1973$, thus diminishing an area of 33 m^2 to 8 m^2 implies
398 savings of $\text{US}\$49325$, comparable to energy costs of 16 years of operation with LED

Accepted Author Manuscript.

399 artificial lighting, which seems an economically and environmental sustainable scenario for
400 FW digestate treatment, even without considering the economical values of sub products
401 reuse.

402 **Conclusions**

403 Food waste digestate with high ammonia concentrations and a C:N:P ratio of 74:74:1
404 showed to have an inhibitory force over a high range of microalgae species. However, *C.*
405 *vulgaris* was able to survive and perform photoaeration and nutrients assimilation when
406 illuminated with solar lighting in control reactors or LED lights (660 nm , $15\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
407 in the LED reactors. LED reactors exhibited a better performance in S-COD removal, with
408 efficiencies of $95.81\pm 0.92\%$, higher than efficiencies of 84.02 ± 4.05 obtained in the control
409 reactors. TAN efficiencies of LED reactors were less than those obtain in the control
410 reactors as a consequence of higher ammonia volatilization at slightly higher pH values. No
411 significant differences between control and LED reactors were found for $\text{PO}_4\text{-P}$ removal
412 efficiencies. Nutrients and microbiological composition of treated FWD suggest that either
413 energetic or agricultural reuses are opportunities for increasing sustainability of FW
414 management. LED Lighting at 660 nm and $15\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ is a cost-effective solution to
415 optimize bioreactors for microalgae-based treatment of highly concentrated digestates and
416 other wastewaters.

417 **Acknowledgments**

418 Authors acknowledge the Minas Gerais State Agency for Research and Development –
419 (FAPEMIG) for funding support through project 694 - FAPEMIG - APQ-02752-14 and

Accepted Author Manuscript.

420 *National Council for Scientific and Technological Development (CNPq)* for the scholarhip
421 141428/2016-3.

422 **References**

- 423 1. Ayre, J. M., Moheimani, N. R., & Borowitzka, M. A. 2017. Growth of microalgae on undiluted
424 anaerobic digestate of piggery effluent with high ammonium concentrations. *Algal Research*, 24,
425 218-226.
- 426 2. Azov, Y., & Goldman, J. C. 1982. Free ammonia inhibition of algal photosynthesis in intensive
427 cultures. *Applied and environmental microbiology*, 43(4), 735-739.
- 428 3. Carvalho, A. P., Silva, S. O., Baptista, J. M., & Malcata, F. X. 2011. Light requirements in
429 microalgal photobioreactors: an overview of biophotonic aspects. *Applied microbiology and*
430 *biotechnology*, 89(5), 1275-1288.
- 431 4. Choi, H. J., & Lee, S. M. 2015. Effect of the N/P ratio on biomass productivity and nutrient removal
432 from municipal wastewater. *Bioprocess and biosystems engineering*, 38(4), 761-766.
- 433 5. Emerson, K., Russo, R. C., Lund, R. E., Thurston, R. V., Zarka, A. 1975 Aqueous Ammonia
434 Equilibrium Calculations: Effects of PH and Temperature. *Journal of the Fisheries Research Board*
435 *of Canada* 32, 2379–2383.
- 436 6. Gujer, W. 2010. Nitrification and me–A subjective review. *Water research*, 44(1), 1-19.
- 437 7. Gutierrez, J., Kwan, T. A., Zimmerman, J. B., & Peccia, J. 2016. Ammonia inhibition in oleaginous
438 microalgae. *Algal Research*, 19, 123-127.
- 439 8. Kang, J., Kwon, G., Nam, J. H., Kim, Y. O., & Jahng, D. 2017. Carbon dioxide stripping from
440 anaerobic digestate of food waste using two types of aerators. *International Journal of*
441 *Environmental Science and Technology*, 14(7), 1397-1408.
- 442 9. Mayers, J. J., Nilsson, A. E., Albers, E., & Flynn, K. J. 2017. Nutrients from anaerobic digestion
443 effluents for cultivation of the microalga *Nannochloropsis* sp.—Impact on growth, biochemical
444 composition and the potential for cost and environmental impact savings. *Algal Research*, 26, 275-
445 286.
- 446 10. Maxwell, D. P., Falk, S., & Huner, N. P. 1995. Photosystem II excitation pressure and development
447 of resistance to photoinhibition (I. light-harvesting complex II abundance and zeaxanthin content in
448 *Chlorella vulgaris*). *Plant Physiology*, 107(3), 687-694.
- 449 11. Mezzomo, N., Saggiorato, A. G., Siebert, R., Tatsch, P. O., Lago, M. C., Hemkemeier, M., ... &
450 Colla, L. M. 2010. Cultivation of microalgae *Spirulina platensis* (*Arthrospira platensis*) from
451 biological treatment of swine wastewater. *Food Science and Technology (Campinas)*, 30(1), 173-
452 178.

Accepted Author Manuscript.

- 453 12. Mohammed, K., Ahammad, Z. S., Sallis, P. J., & Mota, C. R. 2013. Optimisation of red light-
454 emitting diodes irradiance for illuminating mixed microalgal culture to treat municipal wastewater.
455 *WIT Transactions on Ecology and the Environment*, 178, 263-270.
- 456 13. Mohammed, K., Ahammad, S. Z., Sallis, P. J., & Mota, C. R. 2014. Energy-efficient stirred-tank
457 photobioreactors for simultaneous carbon capture and municipal wastewater treatment. *Water*
458 *Science and Technology*, 69(10), 2106-2112.
- 459 14. Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and
460 crop growth: a review. *Engineering in Life Sciences*, 12(3), 242-257.
- 461 15. Park, J. B. K., & Craggs, R. J. 2011. Nutrient removal in wastewater treatment high rate algal ponds
462 with carbon dioxide addition. *Water Science and Technology*, 63(8), 1758-1764.
- 463 16. Park, J. B. K., Craggs, R. J., & Shilton, A. N. 2011. Wastewater treatment high rate algal ponds for
464 biofuel production. *Bioresource technology*, 102(1), 35-42.
- 465 17. Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. 2012. Standard methods for the
466 examination of water and wastewater. American Public Health Association, American Water Works
467 Association, and Water Environment Federation.
- 468 18. Sheets, J. P., Yang, L., Ge, X., Wang, Z., & Li, Y. 2015. Beyond land application: emerging
469 technologies for the treatment and reuse of anaerobically digested agricultural and food waste. *Waste*
470 *Management*, 44, 94-115.
- 471 19. Sorokin, C., & Krauss, R. W. (1958). The Effects of Light Intensity on the Growth Rates of Green
472 Algae. *Plant physiology*, 33(2), 109.
- 473 20. Tricolici, O., Bumbac, C., Patroescu, V., & Postolache, C. 2014. Dairy wastewater treatment using
474 an activated sludge–microalgae system at different light intensities. *Water Science and*
475 *Technology*, 69(8), 1598-1605.
- 476 21. Yan, C., Zhang, L., Luo, X., & Zheng, Z. 2013. Effects of various LED light wavelengths and
477 intensities on the performance of purifying synthetic domestic sewage by microalgae at different
478 influent C/N ratios. *Ecological engineering*, 51, 24-32.
- 479 22. van der Steen, P., Rahsilawati, K., Rada-Ariza, A. M., Lopez-Vazquez, C. M., & Lens, P. N. 2015. A
480 new photo-activated sludge system for nitrification by an algal-bacterial consortium in a photo-
481 bioreactor with biomass recycle. *Water Science and Technology*, 72(3), 443-450.
- 482 23. Von Sperling, M. 2002. Lagoas de estabilização. In *Lagoas de estabilização*. DESA/UFMG.
- 483 24. Wu, H. 2016. Effect of Different Light Qualities on Growth, Pigment Content, Chlorophyll
484 Fluorescence, and Antioxidant Enzyme Activity in the Red Alga *Pyropia haitanensis* (Bangiales,
485 Rhodophyta). *BioMed research international*, 2016.

Accepted Author Manuscript.

- 486 25. Zhao, Y., Wang, J., Zhang, H., Yan, C., & Zhang, Y. 2013. Effects of various LED light
487 wavelengths and intensities on microalgae-based simultaneous biogas upgrading and digestate
488 nutrient reduction process. *Bioresource technology*, 136, 461-468