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# Electro-separation of microalgal culture from wastewater

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## ***Abstract***

For further applications of microalgae such as bio-products, microalgal harvesting from its culture medium (e.g. wastewater) must be studied. This becomes more essential when investigating whether or not cells can stay viable to be recycled into the system. Microalgae culture, wastewater, and a mixture of both were separately electrocoagulated at wastewater Chemical Oxygen Demand ranging 66-2700 mg.l<sup>-1</sup> and biomass dry weights between 1-8 g.l<sup>-1</sup>. The mixed culture contained species of *C. Vulgaris*, *S. Obliquus*, *B. Braunii*, *B. Sudeticus*, and *A. Falcatus*, since mixed culture technique can reduce the expenses in industrial scales by eliminating the costly sterilization strategies necessary to avoid contamination. The mixed samples were successfully separated with the efficiencies between 44-87% and 70-80% at different Chemical Oxygen Demand and biomass dry weights, respectively.

In addition, it was shown that growth elements of carbon and nitrogen, although at lower rates, were consumed confirming the viability of the cells after electrocoagulation. The consumption rates for electrocoagulated samples were smaller than non-electrocoagulated samples only by 16, 12, and 31% in carbon, nitrate and ammonium concentrations,

24 respectively. According to the obtained results electrical separation of microalgae could  
25 effectively harvest microalgae from wastewater without affecting the viability of the  
26 biomass.

27 **Key words:** Electrocoagulation, Harvest, Microalgae, Mixed culture, Wastewater

## 28 **1. Introduction**

29 Renewable energy and treatment of wastewater are two topics of immense importance in the  
30 current century. In one hand, the concerns over fossil fuels consumption grow every day, and  
31 renewable biofuels seem to be a promising substitute. However, oil crops and waste oil  
32 cannot provide the current demand for fuel, and microalgae can be a significant aid as  
33 feedstock for biofuel production (Chisti 2007, Christenson and Sims 2011). Microalgae can  
34 provide human with a more promising source for biofuel, bio-methane, and many other  
35 currently oil-based materials like bio-plastic and fertilizers, needless to mention the cosmetic,  
36 medical, and food industries that can benefit from microalgae bioproducts (Chiellini, Cinelli  
37 et al. 2008, Roeselers, Van Loosdrecht et al. 2008, Barros, Gonçalves et al. 2015).

38 On the other hand, the shortage of fresh water has led to universal attempts to find sustainable  
39 water management strategies. Bio-treatment using microalgae has received attention since the  
40 removal of the nutrients is less expensive and more environmental friendly compared to  
41 conventional chemical methods (Hoffmann 1998, Christenson and Sims 2011, Abdel-Raouf,  
42 Al-Homaidan et al. 2012)

43 As a result, it would be a promising idea to use microalgae to treat the wastewater of its  
44 nutrients and generate biofuel and other bioproducts. Nevertheless, the most costly stage of  
45 microalgae-based technology would be its harvesting from the liquid phase reaching to 20-  
46 60% of the total cost (Sander and Murthy 2010, Nguyen, Le et al. 2019). Many strategies,

47 including centrifugation, coagulation, ultrasonic, pH change, filtration, etc., have been  
48 applied to separate the microalgae from the liquid phase (Fayad, Yehya et al. 2017, Nguyen,  
49 Le et al. 2019). Electrocoagulation (EC) is one of the most widely applied strategies to  
50 harvest microalgae (Gao, Yang et al. 2010, Uduman, Qi et al. 2010) and to treat different  
51 wastewater (Gao, Yang et al. 2010). Researches have reported up to 95% of the microalgae  
52 removal by electrocoagulation (Uduman, Qi et al. 2010). Furthermore, electrocoagulation has  
53 been successfully applied to treat various wastewater with perfect efficiencies (Sahu,  
54 Mazumdar et al. 2014). In these studies, microalgae was separated mainly from growth  
55 medium dissolved in water, and other separation mediums like wastewater have been rarely  
56 discussed (Udom, Zaribaf et al. 2013). In one of the very rare studies on algae harvesting  
57 from wastewater, the chemical coagulation was applied as the harvesting technique (Udom,  
58 Zaribaf et al. 2013). In addition, one major bottleneck in microalgae application is the low  
59 productivity of the culture in terms of product formation and biomass. Besides, many  
60 microalgal products are secondary metabolites which are produced at the cost of growth  
61 limitation. If these metabolites can be removed continuously from the cells, the biomass can  
62 be re-used to produce the high-value compounds (Hejazi and Wijffels 2004). Therefore, the  
63 viability of cells at different stages of industrial operations can be very important. This must  
64 be added to the fact that the viable biomass can always be recycled and used as inoculum for  
65 the next growth generation. However, there have rarely been studies to investigate the effect  
66 of harvesting techniques on the cell viabilities. In one study, the chemical coagulation seems  
67 to have had no effect on the cells viability (Papazi, Makridis et al. 2010), although no  
68 investigation has been found to inspect electrocoagulation for similar results.

69 The harvesting of a mixed culture of microalgae from wastewater using electrocoagulation  
70 has been rarely focused in literature. In addition, there has been no study to inspect the  
71 viability of microalgal cells after electrocoagulation. Therefore, this study aims to investigate

72 the efficiency of EC for harvesting a mixed culture of microalgae from an industrial  
73 wastewater medium. In addition, the effect of EC on the microalgal growth was investigated  
74 through a series of viability experiments.

75

## 76 **2. Materials and Methods**

### 77 ***2.1. Microalgae medium and cultivation***

78 A mixed culture containing *C. Vulgaris*, *S. Obliquus*, *B. Braunii*, *B. Sudeticus*, and *A.*  
79 *Falcatus* was prepared and inoculated into a 4-liter cylindrical photobioreactor (PBR) filled  
80 with autoclaved 3N-BBM+V (modified Bold Basal Medium with 3-fold Nitrogen and  
81 Vitamins) upto 3.5 liters. The 3N-BBM+V medium consisted of macro-nutrients: 0.75 g  
82 NaNO<sub>3</sub>, 0.025 g CaCl<sub>2</sub>.2H<sub>2</sub>O, 0.075 g MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.075 g K<sub>2</sub>HPO<sub>4</sub>.3H<sub>2</sub>O, 0.175 g  
83 KH<sub>2</sub>PO<sub>4</sub>, 0.025 g NaCl and micro-nutrients: 4.5 mg Na<sub>2</sub>EDTA, 0.582 mg FeCl<sub>3</sub>.6H<sub>2</sub>O, 0.246  
84 mg MnCl<sub>2</sub>.4H<sub>2</sub>O, 0.03 mg ZnCl<sub>2</sub>, 0.012 mg CoCl<sub>2</sub>.6H<sub>2</sub>O, 0.024 mg Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O, 1.2 mg  
85 Thiamine hydrochloride as well as 0.01 mg Cyanocobalamin, per liter of DI water (Guo and  
86 Tong 2014). All chemicals were purchased from Sigma–Aldrich (Singapore). The PBR was  
87 illuminated using four 13W 6700K florescent lamps and aerated with a mixed flow of air and  
88 CO<sub>2</sub> (1.75 LPM air and its 5% CO<sub>2</sub> flow) with an aeration rate of 0.5 vvm. In addition to the  
89 air flow, the content of the culture flask was magnetically stirred to provide good mixing  
90 under room temperature. When a dry weight (DW) of 2 g.l<sup>-1</sup> was obtained, the algal culture  
91 was used for the subsequent electrocoagulation. The required microalgae were diluted or  
92 concentrated depending on the desired DW values using distilled water or centrifugation,  
93 respectively.

### 94 ***2.2. Wastewater***

95 A food industry wastewater was used with an initial Chemical Oxygen Demand (COD) of  
96 20000 mg.l<sup>-1</sup>. This concentration was later diluted to obtain the desired COD values for the  
97 harvesting experiments using distilled water. Although the set-up was not aimed to perform  
98 in a sterile condition, the wastewater was autoclaved in order to make sure that no other  
99 micro-organism existed at the start of the experiment.

### 100 ***2.3.Electrocoagulation cell***

101 The EC cell consisted of a 250-mililiter beaker equipped with Aluminum electrodes  
102 connected to a DC Power supply. The sample volume was 200 milliliters, and EC time was 5  
103 min. Each sample was left to settle for 5 min before sampling. The whole sample, without  
104 modification, was later left for further microalgal growth. The current density for all  
105 experiments was 250 A.m<sup>-2</sup>, and the interelectrode distance was 1cm. The EC experiments  
106 were performed for microalgae (MIC), wastewater (WW), and the mixture of both (MWW).  
107 In case of microalgae and wastewater mix (MWW) the ratio was 1:9, respectively. In pure  
108 microalgae and pure wastewater experiments, the distilled water was replaced with similar  
109 ratios. Each EC experiment was performed in duplicates to ensure the reproducibility of the  
110 results.

### 111 ***2.4.Analytical Methods***

112 For each set of harvesting experiments, the Chemical Oxygen Demand COD was measured  
113 before and after the electrocoagulation was run. The COD was measured using dichromate  
114 according to standard methods (Baird, Bridgewater et al. 2012). All tests were performed  
115 three times and an average value was reported.

116 The dry weight (DW) was reported by measuring the difference between the weights of a  
117 dried filter before and after addition of 5 milliliters of sample. To dry the filter before and

118 after microalgae addition, it was kept in an oven at 105 °C for a day and then cooled in a  
119 desiccator (Baird, Bridgewater et al. 2012).

120 For determining the dissolved nitrogen, the ammonium and nitrate tests were measured by  
121 phenate and spectrophotometric methods, respectively (Baird, Bridgewater et al. 2012). All  
122 tests were performed three times and an average value was reported.

### 123 **3. Results**

#### 124 ***3.1. The effect of wastewater concentration***

125 The results of COD removal by electrocoagulation based on varying initial wastewater COD  
126 concentrations for WW and MWW are depicted in Figure 1. In WW and MWW experiments,  
127 with higher COD values the removal efficiency started to decrease. In WW experiments, the  
128 recovery values for the CODs of 82, 266, 543, 827, and 2748 mg.l<sup>-1</sup> were 100, 88, 87, 67, and  
129 39%, respectively.

130 In addition, for MWW experiments, the recovery values were 87, 79, 77, 50, and 44%,  
131 respectively. To ensure consistency of the resulted trend for removal efficiency through COD  
132 results, Optical Density (OD) of the samples before and after the EC run were also measured  
133 and recovery was calculated in terms of OD values (Zongo, Maiga et al. 2009, De Godos,  
134 Guzman et al. 2011) (See supplementary file).

#### 135 ***3.2. The effect of microalgal concentration***

136 When the initial dry weight of microalgae was changed, the recovery rate maintained at high  
137 values. These results have been illustrated in the Figure 2. The initial wastewater COD was  
138 measured to be between 193 and 263 mg.l<sup>-1</sup> and after the EC run, the COD removal for WW  
139 varied between 74 and 92% (not shown in the graph). For microalgae, the initial dry weights

140 were 1, 2, 4, and 8 g.l<sup>-1</sup>. The removal efficiencies for MIC were 96, 89, 76, and 90% for 1, 2,  
141 4, and 8 g.l<sup>-1</sup>.

142 The MWW only had a slight change, since no big drop in removal of microalgae culture had  
143 occurred. Except for microalgal cell density of 1 g.l<sup>-1</sup>, where the removal was 68% the three  
144 other cell concentrations were measured to be 80%. Here, too, OD of the samples were also  
145 measured and patterns were compared with the data from COD analysis (refer to  
146 supplementary data).

### 147 ***3.3. The viability tests***

148 Two separate sets of microalgae samples, electrocoagulated (EC) and non-electrocoagulated  
149 (non-EC), were studied for the consumption of important nutrients for a 7-day period. All  
150 growth conditions were as described above. To study the nitrogen consumption, ammonium  
151 and nitrate tests were performed on daily basis, and the COD test was applied to study the  
152 consumption of carbonic compounds. The results of COD, nitrate, and ammonium tests can  
153 be found in figures 3, 4, and 5, respectively. Figure 3 shows that carbon sources in the non-  
154 EC sample were consumed at a rate of 17.72 mg.l<sup>-1</sup>.day<sup>-1</sup> while it was consumed at the rate of  
155 14.89 mg.l<sup>-1</sup>.day<sup>-1</sup> in EC sample. In other words, the COD was removed at least 60% in both  
156 EC and non-EC samples.

157 On the other hand, the consumption of nitrate was measured to investigate consumption of  
158 the nitrogen source for growth. The results are depicted in Figure 4. The nitrate consumption  
159 rates were measured to be 2.52 and 2.21 mg.l<sup>-1</sup>.day<sup>-1</sup> for non-EC and EC samples,  
160 respectively. Based on the initial nitrogen concentrations, dissolved N was removed by 35-  
161 40% from the mediums.

162 Since ammonium is a different nitrogen source present in wastewater, its consumption rate  
163 was also monitored. Figure 5 shows the ammonium consumption within a 7-day period.  
164 While ammonium consumption rate is  $0.638 \text{ mg.l}^{-1}.\text{day}^{-1}$  for non-EC sample, it was  $0.440$   
165  $\text{mg.l}^{-1}.\text{day}^{-1}$  for the EC sample. Results can be interpreted as the removal of 15-21% of  
166 ammonium from the mediums.

#### 167 **4. Discussion**

168 Although electrocoagulation has been applied for years even at industrial scale for  
169 wastewater treatment and recently for biomass separation, the involved mechanisms have  
170 been seriously argued. The current theory states that EC involves several sequent stages  
171 (Moreno-Casillas, Cocke et al. 2007): first, the metal ions are generated. Then, the metal ions  
172 hydrolysis occurs and metal hydroxides and polyhydroxides form. Water is simultaneously  
173 electrolyzed producing small bubbles of oxygen at the anode and hydrogen at the cathode.  
174 Next, the particles are destabilized, the emulsions are broken and then come together to  
175 aggregate and form flocs. Finally, chemical reactions and precipitation can occur including  
176 hydroxyl ions forming precipitate with particles. These mechanisms, though affected by  
177 biomass/wastewater concentration, individually or collectively provided both colloidal  
178 (wastewater) and biological (microalgae) separations.

##### 179 ***4.1. The effect of wastewater concentration***

180 At constant conditions like current density and time, the falling trend of removal efficiency  
181 with higher initial concentration was observed which is in agreement with the results in other  
182 studies (Aoudj, Khelifa et al. 2010). The removal efficiency is quite comparable to many  
183 studies in the literature (Olguín 2012, Fernandes, Pacheco et al. 2015), although the  
184 efficiencies often vary widely from one study to another, since the exact composition of  
185 wastewater complicates the comparison. In one study, for example, on the pulp and paper

186 industry effluent, with an initial COD of  $620 \text{ mg.l}^{-1}$ , the COD removal efficiency at the same  
187 current density was reported to be around 50% (Sridhar, Sivakumar et al. 2011). Apart from  
188 the chemical composition, the 3-centimeter interelectrode distance has decreased the  
189 efficiency compared to the current study value where the electrode gap was 1 cm. With  
190 increasing the distance, a decrease in the amount of anode dissolution will occur, and the ions  
191 need to transfer a longer distance for interaction to form flocs. Thus, with less flocs  
192 formation, COD removal will decrease (Khandegar and Saroha 2012). One study used natural  
193 flocculants of Ecotan and Tanfloc to harvest microalgal culture from a pre-treated urban  
194 wastewater set-up. The optimal biomass recovery was reported to be 92 and 90% for Ecotan  
195 and Tanfloc, respectively. A dose amounts of 10 and  $50 \text{ mg.l}^{-1}$  were, respectively, used for  
196 these two natural flocculants (Gutiérrez, Passos et al. 2015). As that study reports, the COD  
197 of the set-up influent was  $250 \text{ mg.l}^{-1}$  on average (Passos, Solé et al. 2013, Gutiérrez, Passos et  
198 al. 2015), which is quite comparable with the WW and MWW results in this study, especially  
199 since no optimization was aimed and practiced here. Yet, in another study on harvesting  
200 bacterial and microalgal cultures from a piggery wastewater, seven different coagulants and  
201 flocculants were tested including two conventional coagulants of  $\text{FeCl}_3$  and  $\text{Fe}_2(\text{SO}_4)_3$ , and  
202 five commercial polymeric flocculants such as Chitosan. The researchers tested different  
203 doses of these chemicals. The best removal efficiencies were generally for  $\text{FeCl}_3$  and  
204  $\text{Fe}_2(\text{SO}_4)_3$ . Efficiencies higher than 90% all occurred for high doses of coagulants/flocculants,  
205 between  $150\text{-}250 \text{ mg.l}^{-1}$ . The wastewater tested here, too, was far less ( $=202 \text{ mg.l}^{-1}$ ) than the  
206 maximum amount of COD that microalgal biomass was introduced to in the current study  
207 (De Godos, Guzman et al. 2011).

208 The decrease in COD removal can be associated to the present compounds. In an EC process,  
209 “the COD may increase” due to the reaction of some compounds such as acids with the metal  
210 ions to form soluble products which remain in the solution. On the other hand, soluble and

211 miscible compounds that do not react with metal ion can completely “keep the COD  
212 unchanged”. However, organic salts can form insoluble compounds with metal hydroxide  
213 which leads to “partial removal of the COD” from the medium. Since these compounds  
214 usually consist the main body of municipal and industrial wastewater (Moreno-Casillas,  
215 Cocke et al. 2007) with higher concentration of such compounds at more concentrated  
216 wastewater, less COD can be removed from the medium accordingly.

#### 217 ***4.2. The effect of microalgal concentration***

218 Except for 8 g.l<sup>-1</sup> sudden increase, the falling pattern was expected due to increase in cell  
219 density. This falling pattern can be associated with the adequacy of metal ions to remove the  
220 excessive algae along with the decrease in the reaction rate in EC process. (Gao, Yang et al.  
221 2010). It was already reported that there is no linear correlation between the concentrations of  
222 microalgae and the removal efficiency (Tenney, Echelberger et al. 1969, De Godos, Guzman  
223 et al. 2011). However, the non-linear correlation between the cell concentration and removal  
224 efficiency may be attributed to algogenic organic matter (AOM). The negative effect of AOM  
225 on coagulation has been addressed before (Zhuang, Wu et al. 2016). On the other hand, the  
226 algae cell itself, in the category of suspended solid particles, can be removed with high  
227 efficiencies due to the in-situ-generated coagulants (Moreno-Casillas, Cocke et al. 2007).  
228 The 8-gram microalgal sample was concentrated using centrifugation of four similar 2-gram  
229 samples in a way that the growth culture medium was removed after being centrifuged and  
230 replaced with and mixed in a fresh growth medium together. Consequently, the AOM in the  
231 four samples had been removed and therefore its negative effect on the coagulation process  
232 had been mitigated.

233 The results obtained from this study are quite comparable with other studies, given the fact  
234 that the cell density in those studies was either much lower than present research (<1 mg.l<sup>-1</sup>)  
235 (Vandamme, Pontes et al. 2011) or reported in cell count (Gao, Yang et al. 2010, Wong, Ho

236 et al. 2017). In one of the rare studies on harvesting microalgae from wastewater, six  
237 chemicals were used to harvest *Chlorella* at both wild and lab-cultured species from  
238 wastewater. These chemicals included two reagents of alum and ferric chloride, cationic  
239 polymer, anionic polymer, and natural polymers. The best removal efficiency was achieved  
240 by ferric chloride and alum in which microalgal culture could be harvested by 93 and 91%  
241 efficiency, respectively. It is worth mentioning that to obtain these efficiencies, 122 mg.l<sup>-1</sup> of  
242 ferric chloride and 140 mg.l<sup>-1</sup> of alum were used (Udom, Zaribaf et al. 2013). These amounts  
243 of additive chloride and sulfate ions yet again bring in the conventional debate over the  
244 benefits of electrocoagulation over coagulation. In addition, in the noted study, no separate  
245 data were provided on the flocculation of the wastewater itself especially because the carbon  
246 source was provided through CO<sub>2</sub> flow. In another study the effect of biomass concentration  
247 on the removal efficiency was tested. In this study, two commercial flocculants, namely  
248 Drewfloc-447 and Chemifloc CV-300, were applied. For both flocculants, almost nothing  
249 happened when the concentration of biomass doubled. On the other hand, when the initial  
250 concentration of biomass was halved, the removal efficiency rose by 50% in Drewfloc-447  
251 case and fell by 12% (De Godos, Guzman et al. 2011). Although, the mixed rising and falling  
252 patterns associated with concentration change have been also observed in the current study,  
253 these patterns are more moderate. This difference seems to be the result of a mixed culture,  
254 since in mentioned work, only a pure culture of *C. Sorokiniana* was investigated.  
255 Results of harvesting at both different biomass and wastewater concentrations show that  
256 although biological features can help decrease or increase the efficiency, in terms of  
257 coagulation both colloidal and biological particles act similarly. These results are perfectly in  
258 accordance with previous studies (Pieterse and Cloot 1997).  
259 For the MWW values, the measures were more uniform. MWW values for recovery  
260 efficiency for all the dry weights, except for 1 g.l<sup>-1</sup>, were measured to be approximately 80%.

261 **4.3. The viability tests**

262 It must be noted that small difference in the initial values of COD in both samples can be due  
263 to the COD reduction that normally occurs due to electro-oxidation, electrocoagulation, etc.  
264 (Moreno-Casillas, Cocke et al. 2007).

265 In one study on the growth of a *Chlorella* on wastewater, the COD was removed by 90% over  
266 the course of 14 days. In addition, 90% of the total nitrogen and 93% of ammonium were  
267 removed at the same interval (Li, Chen et al. 2011). Since the cell concentration in both  
268 studies were almost similar, the COD removal can be attributed to the difference between the  
269 microalgal species. While *C. Vulgaris* is only one of the microalgae species present in the  
270 current study, in the mentioned research the microalgal medium mainly contained *Chlorella*  
271 which is known to be a very good mixotrophic, meaning that it can feed both on CO<sub>2</sub> and  
272 organic sources (Martínez, Camacho et al. 1997). As a result, the cell dry weight in that study  
273 has multiplied by a factor of 12 from 0.1 to 1.2 g.l<sup>-1</sup> within the experiment time (Li, Chen et  
274 al. 2011).

275 In another study, in which cultivation of bacterial and microalgal biomass was investigated  
276 on a piggery wastewater, the COD was removed by a range between 49 and 78% for  
277 *Chlorella* consortium, *S. obliquus*, *Chlorococcum* sp., and *C. sorokiniana* species. In  
278 addition, the consumption of N-NH<sub>4</sub><sup>+</sup> was also investigated. The N-NH<sub>4</sub><sup>+</sup> removal was  
279 reported to be between 77 and 81% (De Godos, Guzman et al. 2011).

280 These data from COD, nitrate and ammonium consumption rates collectively states that  
281 although the consumption rates slightly differ from each other, yet confirm the consumption  
282 of carbon and nitrogen sources meaning that a great number of microalgae are viable and  
283 growing. In addition, the slight reduction in consumption rates of these sources may indicate  
284 a part of biomass culture has been inactivated due to oxidative stress, production of harmful

285 oxidants, and/or irreversible membrane permeabilization caused by EC (Wei, Elektorowicz et  
286 al. 2011). The confirmation of biomass viability in the current study is in agreement with  
287 previous work on bacteria (Wei, Elektorowicz et al. 2011). Studies show that other methods  
288 of biomass harvesting can lead to similar conclusions with cell viability. In one case,  
289 researchers used three methods of centrifugation to harvest 9 different species of microalgae.  
290 The most vulnerable species in that study suffered only from 12% of biomass viability  
291 (Heasman, Diemar et al. 2000).

## 292 **5. Conclusion**

293 In this study, a mixed microalgal culture was successfully harvested from a wastewater  
294 medium with high recovery efficiency. These recovery efficiencies continued to maintain at  
295 high rates even at high concentrations of wastewater and microalgae. The results showed that  
296 the growth nutrients represented by COD, ammonium and nitrate were all consumed,  
297 although slightly smaller than non-electrocoagulated samples, in the course of a 7-day re-  
298 culturing after the electrocoagulation. These results confirm that cells were viable after the  
299 harvesting process. Therefore, electrocoagulation can be used to harvest microalgae from  
300 wastewater without the risk of disrupting of the microalgal cells.

## 301 **6. Conflict of Interest**

302 This research did not receive any specific grant from funding agencies in the public,  
303 commercial, or not-for-profit sectors.

## 304 **7. References**

305 Abdel-Raouf, N., A. Al-Homaidan and I. Ibraheem (2012). "Microalgae and wastewater  
306 treatment." *Saudi journal of biological sciences* 19(3): 257-275.  
307 Aoudj, S., A. Khelifa, N. Drouiche, M. Hecini and H. Hamitouche (2010).  
308 "Electrocoagulation process applied to wastewater containing dyes from textile industry."  
309 *Chemical Engineering and Processing: Process Intensification* 49(11): 1176-1182.

310 Baird, R. B., L. Bridgewater, L. S. Clesceri, A. D. Eaton and E. W. Rice (2012). Standard  
311 methods for the examination of water and wastewater, American public health association.

312 Barros, A. I., A. L. Gonçalves, M. Simões and J. C. Pires (2015). "Harvesting techniques  
313 applied to microalgae: a review." *Renewable and Sustainable Energy Reviews* 41: 1489-  
314 1500.

315 Chiellini, E., P. Cinelli, V. I. Ilieva and M. Martera (2008). "Biodegradable thermoplastic  
316 composites based on polyvinyl alcohol and algae." *Biomacromolecules* 9(3): 1007-1013.

317 Chisti, Y. (2007). "Biodiesel from microalgae." *Biotechnology advances* 25(3): 294-306.

318 Christenson, L. and R. Sims (2011). "Production and harvesting of microalgae for wastewater  
319 treatment, biofuels, and bioproducts." *Biotechnology advances* 29(6): 686-702.

320 De Godos, I., H. O. Guzman, R. Soto, P. A. García-Encina, E. Becares, R. Muñoz and V. A.  
321 Vargas (2011). "Coagulation/flocculation-based removal of algal–bacterial biomass from  
322 piggery wastewater treatment." *Bioresource technology* 102(2): 923-927.

323 Fayad, N., T. Yehya, F. Audonnet and C. Vial (2017). "Harvesting of microalgae *Chlorella*  
324 *vulgaris* using electro-coagulation-flocculation in the batch mode." *Algal Research* 25: 1-11.

325 Fernandes, A., M. Pacheco, L. Ciríaco and A. Lopes (2015). "Review on the electrochemical  
326 processes for the treatment of sanitary landfill leachates: present and future." *Applied*  
327 *Catalysis B: Environmental* 176: 183-200.

328 Gao, S., J. Yang, J. Tian, F. Ma, G. Tu and M. Du (2010). "Electro-coagulation–flotation  
329 process for algae removal." *Journal of Hazardous Materials* 177(1-3): 336-343.

330 Guo, Z. and Y. W. Tong (2014). "The interactions between *Chlorella vulgaris* and algal  
331 symbiotic bacteria under photoautotrophic and photoheterotrophic conditions." *Journal of*  
332 *applied phycology* 26(3): 1483-1492.

333 Gutiérrez, R., F. Passos, I. Ferrer, E. Uggetti and J. García (2015). "Harvesting microalgae  
334 from wastewater treatment systems with natural flocculants: effect on biomass settling and  
335 biogas production." *Algal research* 9: 204-211.

336 Heasman, M., J. Diemar, W. O'connor, T. Sushames and L. Foulkes (2000). "Development of  
337 extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve  
338 molluscs—a summary." *Aquaculture Research* 31(8-9): 637-659.

339 Hejazi, M. A. and R. H. Wijffels (2004). "Milking of microalgae." *TRENDS in*  
340 *Biotechnology* 22(4): 189-194.

341 Hoffmann, J. P. (1998). "Wastewater treatment with suspended and nonsuspended algae."  
342 *Journal of Phycology* 34(5): 757-763.

343 Khandegar, V. and A. K. Saroha (2012). "Electrochemical treatment of distillery spent wash  
344 using aluminum and iron electrodes." *Chinese Journal of Chemical Engineering* 20(3): 439-  
345 443.

346 Li, Y., Y.-F. Chen, P. Chen, M. Min, W. Zhou, B. Martinez, J. Zhu and R. Ruan (2011).  
347 "Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal  
348 wastewater for nutrient removal and biodiesel production." *Bioresource technology* 102(8):  
349 5138-5144.

350 Martínez, M. E., F. Camacho, J. Jiménez and J. Espinola (1997). "Influence of light intensity  
351 on the kinetic and yield parameters of *Chlorella pyrenoidosa* mixotrophic growth." *Process*  
352 *Biochemistry* 32(2): 93-98.

353 Moreno-Casillas, H. A., D. L. Cocke, J. A. Gomes, P. Morkovsky, J. Parga and E. Peterson  
354 (2007). "Electrocoagulation mechanism for COD removal." *Separation and purification*  
355 *Technology* 56(2): 204-211.

356 Nguyen, T. D. P., T. V. A. Le, P. L. Show, T. T. Nguyen, M. H. Tran, T. N. T. Tran and S. Y.  
357 Lee (2019). "Bioflocculation formation of microalgae-bacteria in enhancing microalgae

358 harvesting and nutrient removal from wastewater effluent." *Bioresource technology* 272: 34-  
359 39.

360 Olguín, E. J. (2012). "Dual purpose microalgae–bacteria-based systems that treat wastewater  
361 and produce biodiesel and chemical products within a Biorefinery." *Biotechnology advances*  
362 30(5): 1031-1046.

363 Papazi, A., P. Makridis and P. Divanach (2010). "Harvesting *Chlorella minutissima* using cell  
364 coagulants." *Journal of applied Phycology* 22(3): 349-355.

365 Passos, F., M. Solé, J. García and I. Ferrer (2013). "Biogas production from microalgae  
366 grown in wastewater: effect of microwave pretreatment." *Applied Energy* 108: 168-175.

367 Pieterse, A. and A. Cloot (1997). "Algal cells and coagulation, flocculation and sedimentation  
368 processes." *Water Science and Technology* 36(4): 111-118.

369 Roeselers, G., M. Van Loosdrecht and G. Muyzer (2008). "Phototrophic biofilms and their  
370 potential applications." *Journal of applied phycology* 20(3): 227-235.

371 Sahu, O., B. Mazumdar and P. Chaudhari (2014). "Treatment of wastewater by  
372 electrocoagulation: a review." *Environmental science and pollution research* 21(4): 2397-  
373 2413.

374 Sander, K. and G. S. Murthy (2010). "Life cycle analysis of algae biodiesel." *The*  
375 *International Journal of Life Cycle Assessment* 15(7): 704-714.

376 Sridhar, R., V. Sivakumar, V. P. Immanuel and J. P. Maran (2011). "Treatment of pulp and  
377 paper industry bleaching effluent by electrocoagulant process." *Journal of hazardous*  
378 *materials* 186(2-3): 1495-1502.

379 Tenney, M. W., W. F. Echelberger, R. G. Schuessler and J. L. Pavoni (1969). "Algal  
380 flocculation with synthetic organic polyelectrolytes." *Applied microbiology* 18(6): 965-971.

381 Udom, I., B. H. Zaribaf, T. Halfhide, B. Gillie, O. Dalrymple, Q. Zhang and S. J. Ergas  
382 (2013). "Harvesting microalgae grown on wastewater." *Bioresource technology* 139: 101-  
383 106.

384 Uduman, N., Y. Qi, M. K. Danquah, G. M. Forde and A. Hoadley (2010). "Dewatering of  
385 microalgal cultures: a major bottleneck to algae-based fuels." *Journal of renewable and*  
386 *sustainable energy* 2(1): 012701.

387 Vandamme, D., S. C. V. Pontes, K. Goiris, I. Foubert, L. J. J. Pinoy and K. Muylaert (2011).  
388 "Evaluation of electro-coagulation–flocculation for harvesting marine and freshwater  
389 microalgae." *Biotechnology and bioengineering* 108(10): 2320-2329.

390 Wei, V., M. Elektorowicz and J. Oleszkiewicz (2011). "Influence of electric current on  
391 bacterial viability in wastewater treatment." *Water research* 45(16): 5058-5062.

392 Wong, Y., Y. Ho, H. Leung, K. Ho, Y. Yau and K. Yung (2017). "Enhancement of *Chlorella*  
393 *vulgaris* harvesting via the electro-coagulation-flotation (ECF) method." *Environmental*  
394 *Science and Pollution Research* 24(10): 9102-9110.

395 Zhuang, L.-L., Y.-H. Wu, V. M. D. Espinosa, T.-Y. Zhang, G.-H. Dao and H.-Y. Hu (2016).  
396 "Soluble algal products (SAPs) in large scale cultivation of microalgae for biomass/bioenergy  
397 production: a review." *Renewable and Sustainable Energy Reviews* 59: 141-148.

398 Zongo, I., A. H. Maiga, J. Wéthé, G. Valentin, J.-P. Leclerc, G. Paternotte and F. Lapique  
399 (2009). "Electrocoagulation for the treatment of textile wastewaters with Al or Fe electrodes:  
400 Compared variations of COD levels, turbidity and absorbance." *Journal of Hazardous*  
401 *Materials* 169(1-3): 70-76.

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