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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 $mg.l^{-1}$ and biomass dry weights between 1-8 $g.l^{-1}$. 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,

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

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

1. Introduction

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

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

As a result, it would be a promising idea to use microalgae to treat the wastewater of its nutrients and generate biofuel and other bioproducts. Nevertheless, the most costly stage of microalgae-based technology would be its harvesting from the liquid phase reaching to 20-60% of the total cost (Sander and Murthy 2010, Nguyen, Le et al. 2019). Many strategies, including centrifugation, coagulation, ultrasonic, pH change, filtration, etc., have been applied to separate the microalgae from the liquid phase (Fayad, Yehya et al. 2017, Nguyen, Le et al. 2019). Electrocoagulation (EC) is one of the most widely applied strategies to harvest microalgae (Gao, Yang et al. 2010, Uduman, Qi et al. 2010) and to treat different wastewater (Gao, Yang et al. 2010). Researches have reported up to 95% of the microalgae removal by electrocoagulation (Uduman, Qi et al. 2010). Furthermore, electrocoagulation has been successfully applied to treat various wastewater with perfect efficiencies (Sahu, Mazumdar et al. 2014). In these studies, microalgae was separated mainly from growth medium dissolved in water, and other separation mediums like wastewater have been rarely discussed (Udom, Zaribaf et al. 2013). In one of the very rare studies on algae harvesting from wastewater, the chemical coagulation was applied as the harvesting technique (Udom, Zaribaf et al. 2013). In addition, one major bottleneck in microalgae application is the low productivity of the culture in terms of product formation and biomass. Besides, many microalgal products are secondary metabolites which are produced at the cost of growth limitation. If these metabolites can be removed continuously from the cells, the biomass can be re-used to produce the high-value compounds (Hejazi and Wijffels 2004). Therefore, the viability of cells at different stages of industrial operations can be very important. This must be added to the fact that the viable biomass can always be recycled and used as inoculum for the next growth generation. However, there have rarely been studies to investigate the effect of harvesting techniques on the cell viabilities. In one study, the chemical coagulation seems to have had no effect on the cells viability (Papazi, Makridis et al. 2010), although no investigation has been found to inspect electrocoagulation for similar results.

The harvesting of a mixed culture of microalgae from wastewater using electrocoagulation has been rarely focused in literature. In addition, there has been no study to inspect the viability of microalgal cells after electrocoagulation. Therefore, this study aims to investigate the efficiency of EC for harvesting a mixed culture of microalgae from an industrial wastewater medium. In addition, the effect of EC on the microalgal growth was investigated through a series of viability experiments.

2. Materials and Methods

2.1. Microalgae medium and cultivation

A mixed culture containing *C. Vulgaris*, *S. Obliquus*, *B. Braunii*, *B. Sudeticus,* and *A. Falcatus* was prepared and inoculated into a 4-liter cylindrical photobioreactor (PBR) filled with autoclaved 3N-BBM+V (modified Bold Basal Medium with 3-fold Nitrogen and Vitamins) upto 3.5 liters. The 3N-BBM+V medium consisted of macro-nutrients: 0.75 g $NaNO_3$, 0.025 g $CaCl_2.2H_2O$, 0.075 g $MgSO_4.7H_2O$, 0.075 g $K_2HPO_4.3H_2O$, 0.175 g KH_2PO_4, 0.025 g NaCl and micro-nutrients: 4.5 mg Na_2EDTA, 0.582 mg $FeCl_3.6H_2O$, 0.246 mg $MnCl_2.4H_2O$, 0.03 mg $ZnCl_2$, 0.012 mg $CoCl_2.6H_2O$, 0.024 mg $Na_2MoO_4.2H_2O$, 1.2 mg Thiamine hydrochloride as well as 0.01 mg Cyanocobalamin, per liter of DI water (Guo and Tong 2014). All chemicals were purchased from Sigma–Aldrich (Singapore). The PBR was illuminated using four 13W 6700K florescent lamps and aerated with a mixed flow of air and CO_2 (1.75 LPM air and its 5% CO_2 flow) with an aeration rate of 0.5 vvm. In addition to the air flow, the content of the culture flask was magnetically stirred to provide good mixing under room temperature. When a dry weight (DW) of 2 $g.l^{-1}$ was obtained, the algal culture was used for the subsequent electrocoagulation. The required microalgae were diluted or concentrated depending on the desired DW values using distilled water or centrifugation, respectively.

2.2. Wastewater

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

2.3.Electrocoagulation cell

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

2.4.Analytical Methods

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

The dry weight (DW) was reported by measuring the difference between the weights of a dried filter before and after addition of 5 milliliters of sample. To dry the filter before and after microalgae addition, it was kept in an oven at 105 °C for a day and then cooled in a desiccator (Baird, Bridgewater et al. 2012).

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

3. Results

3.1. The effect of wastewater concentration

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

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

3.2. The effect of microalgal concentration

When the initial dry weight of microalgae was changed, the recovery rate maintained at high values. These results have been illustrated in the Figure 2. The initial wastewater COD was measured to be between 193 and 263 $mg.l^{-1}$ and after the EC run, the COD removal for WW varried between 74 and 92% (not shown in the graph). For microalgae, the initial dry weights were 1, 2, 4, and 8 $g.l^{-1}$.The removal efficiencies for MIC were 96, 89, 76, and 90% for 1, 2, 4, and 8 $g.l^{-1}$.

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

3.3.The viability tests

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

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

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

4. Discussion

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

4.1. The effect of wastewater concentration

At constant conditions like current density and time, the falling trend of removal efficiency with higher initial concentration was observed which is in agreement with the results in other studies (Aoudj, Khelifa et al. 2010). The removal efficiency is quite comparable to many studies in the literature (Olguín 2012, Fernandes, Pacheco et al. 2015), although the efficiencies often vary widely from one study to another, since the exact composition of wastewater complicates the comparison. In one study, for example, on the pulp and paper industry effluent, with an initial COD of 620 mg.l^{-1}, the COD removal efficiency at the same current density was reported to be around 50% (Sridhar, Sivakumar et al. 2011). Apart from the chemical composition, the 3-centimeter interelectrode distance has decreased the efficiency compared to the current study value where the electrode gap was 1 cm. With increasing the distance, a decrease in the amount of anode dissolution will occur, and the ions need to transfer a longer distance for interaction to form flocs. Thus, with less flocs formation, COD removal will decrease (Khandegar and Saroha 2012). One study used natural flocculants of Ecotan and Tanfloc to harvest microalgal culture from a pre-treated urban wastewater set-up. The optimal biomass recovery was reported to be 92 and 90% for Ecotan and Tanfloc, respectively. A dose amounts of 10 and 50 mg.l^{-1} were, respectively, used for these two natural flocculants (Gutiérrez, Passos et al. 2015). As that study reports, the COD of the set-up influent was 250 mg.l^{-1} on average (Passos, Solé et al. 2013, Gutiérrez, Passos et al. 2015), which is quite comparable with the WW and MWW results in this study, especially since no optimization was aimed and practiced here. Yet, in another study on harvesting bacterial and microalgal cultures from a piggery wastewater, seven different coagulants and flocculants were tested including two conventional coagulants of $FeCl_3$ and $Fe_2(SO_4)_3$, and five commercial polymeric flocculants such as Chitosan. The researchers tested different doses of these chemicals. The best removal efficiencies were generally for $FeCl_3$ and $Fe_2(SO_4)_3$. Efficiencies higher than 90% all occurred for high doses of coagulants/flocculants, between 150-250 mg.l^{-1}. The wastewater tested here, too, was far less (=202 mg.l^{-1}) than the maximum amount of COD that microalgal biomass was introduced to in the current study (De Godos, Guzman et al. 2011).

The decrease in COD removal can be associated to the present compounds. In an EC process, "the COD may increase" due to the reaction of some compounds such as acids with the metal ions to form soluble products which remain in the solution. On the other hand, soluble and miscible compounds that do not react with metal ion can completely “keep the COD unchanged”. However, organic salts can form insoluble compounds with metal hydroxide which leads to “partial removal of the COD” from the medium. Since these compounds usually consist the main body of municipal and industrial wastewater (Moreno-Casillas, Cocke et al. 2007) with higher concentration of such compounds at more concentrated wastewater, less COD can be removed from the medium accordingly.

4.2. The effect of microalgal concentration

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

The results obtained from this study are quite comparable with other studies, given the fact that the cell density in those studies was either much lower than present research (<1 $mg.l^{-1}$) (Vandamme, Pontes et al. 2011) or reported in cell count (Gao, Yang et al. 2010, Wong, Ho et al. 2017). In one of the rare studies on harvesting microalgae from wastewater, six chemicals were used to harvest *Chlorella* at both wild and lab-cultured species from wastewater. These chemicals included two reagents of alum and ferric chloride, cationic polymer, anionic polymer, and natural polymers. The best removal efficiency was achieved by ferric chloride and alum in which microalgal culture could be harvested by 93 and 91% efficiency, respectively. It is worth mentioning that to obtain these efficiencies, 122 $mg.l^{-1}$ of ferric chloride and 140 $mg.l^{-1}$ of alum were used (Udom, Zaribaf et al. 2013). These amounts of additive chloride and sulfate ions yet again bring in the conventional debate over the benefits of electrocoagulation over coagulation. In addition, in the noted study, no separate data were provided on the flocculation of the wastewater itself especially because the carbon source was provided through CO_2 flow. In another study the effect of biomass concentration on the removal efficiency was tested. In this study, two commercial flocculants, namely Drewfloc-447 and Chemifloc CV-300, were applied. For both flocculants, almost nothing happened when the concentration of biomass doubled. On the other hand, when the initial concentration of biomass was halved, the removal efficiency rose by 50% in Drewfloc-447 case and fell by 12% (De Godos, Guzman et al. 2011). Although, the mixed rising and falling patterns associated with concentration change have been also observed in the current study, these patterns are more moderate. This difference seems to be the result of a mixed culture, since in mentioned work, only a pure culture of *C. Sorokiniana* was investigated.

Results of harvesting at both different biomass and wastewater concentrations show that although biological features can help decrease or increase the efficiency, in terms of coagulation both colloidal and biological particles act similarly. These results are perfectly in accordance with previous studies (Pieterse and Cloot 1997).

For the MWW values, the measures were more uniform. MWW values for recovery efficiency for all the dry weights, except for 1 $g.l^{-1}$, were measured to be approximately 80%.

4.3. The viability tests

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

In one study on the growth of a *Chlorella* on wastewater, the COD was removed by 90% over the course of 14 days. In addition, 90% of the total nitrogen and 93% of ammonium were removed at the same interval (Li, Chen et al. 2011). Since the cell concentration in both studies were almost similar, the COD removal can be attributed to the difference between the microalgal species. While *C. Vulgaris* is only one of the microalgae species present in the current study, in the mentioned research the microalgal medium mainly contained *Chlorella* which is known to be a very good mixotrophic, meaning that it can feed both on CO_2 and organic sources (Martínez, Camacho et al. 1997). As a result, the cell dry weight in that study has multiplied by a factor of 12 from 0.1 to 1.2 $g.l^{-1}$ within the experiment time (Li, Chen et al. 2011).

In another study, in which cultivation of bacterial and microalgal biomass was investigated on a piggery wastewater, the COD was removed by a range between 49 and 78% for *Chlorella* consortium*, S. obliquus, Chlorococcum sp., and C. sorokiniana* species. In addition, the consumption of $N\text{-}NH_4^+$ was also investigated. The $N\text{-}NH_4^+$ removal was reported to be between 77 and 81% (De Godos, Guzman et al. 2011).

These data from COD, nitrate and ammonium consumption rates collectively states that although the consumption rates slightly differ from each other, yet confirm the consumption of carbon and nitrogen sources meaning that a great number of microalgae are viable and growing. In addition, the slight reduction in consumption rates of these sources may indicate a part of biomass culture has been inactivated due to oxidative stress, production of harmful oxidants, and/or irreversible membrane permeabilization caused by EC (Wei, Elektorowicz et al. 2011). The confirmation of biomass viability in the current study is in agreement with previous work on bacteria (Wei, Elektorowicz et al. 2011). Studies show that other methods of biomass harvesting can lead to similar conclusions with cell viability. In one case, researchers used three methods of centrifugation to harvest 9 different species of microalgae. The most vulnerable species in that study suffered only from 12% of biomass viability (Heasman, Diemar et al. 2000).

5. Conclusion

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

6. Conflict of Interest

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

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