Microalgae harvesting and cell disruption: a preliminary evaluation of the technology electroflotation by alternating current
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ABSTRACT

Some species of microalgae have high productivity and lipid content, which makes them good candidates for biodiesel production. Biomass separation and cell disruption are important steps in biodiesel production from microalgae. In this work, we explored the fundamentals of electroflotation by alternating current (EFAC) with non-consumable electrodes to simultaneously harvest microalgae and disrupt cells from mixed microalgae obtained from waste stabilization ponds. The harvesting efficiency was evaluated using chlorophyll-a and turbidity, which reached removals of 99% and 95%, respectively, during a batch time of 140 min. Cell disruption was evaluated using lipid extraction, and the best results were achieved with a batch time of 140 min, which resulted in a 14% yield. Therefore, EFAC was shown to be an attractive potential technology for simultaneous microalgal harvesting and cell disruption.

INTRODUCTION

Microalgae have the potential to generate several types of biofuels, including methane, through the anaerobic digestion of algal biomass (Spolaore et al. 2006), biohydrogen produced from photosynthetic microorganisms (Ghirardi et al. 2000), and biodiesel derived from microalgal oil (Gavrilescu & Chisti 2005).

Biodiesel production using microalgae, compared with other culture types, has several advantages, such as the capacity for year-round production, high growth rate, low water demand, ability to utilize a wide variety of water sources (fresh, brackish, seawater, and wastewater), ability to avoid arable land used to produce food, and production of valuable co-products (Brennan & Owende 2010). The use of wastewater in the cultivation of microalgae has the following advantages: (1) some species are efficient at removing nitrogen, phosphorus, and metallic ions, resulting in combined microalgal cultivation and wastewater treatment, which significantly improves the environmental benefits of the process; (2) costs are reduced with nitrogen and phosphorus because they are usually found in wastewater (Brennan & Owende 2010); and (3) less water is used (Mata et al. 2010).

Some authors affirm that the production of biofuels combined with wastewater treatment has more plausible commercial application in the short term, since it provides a pathway for removing contaminants from wastewater, while promoting the production of biomass for biofuel production (Brennan & Owende 2010). For biodiesel production from microalgae, the biomass has to be separated, and lipids from the cytoplasm have to become available, which requires cell disruption (Rawat et al. 2015). The primary methods to pretreat are autoclaving, ultrasonication, and microwaving as well as treatments with acids, bases, enzymes, or osmotic shocks (Halim et al. 2012). According to Lee et al. (2010), although little scientific research has been done concerning pretreatment methods, this step cannot be neglected because extraction efficiency increases with the degree of cell disruption.

Separation processes that can be utilized for microalgal harvesting include electrolytic processes, centrifugation,
filtration, and flotation or sedimentation assisted by chemical coagulation. Conventional electrolytic chemical-based processes typically involve three reactions. First, metal ions, which are considered effective coagulants, are liberated at the anode by electrolytic oxidation. Simultaneously, oxygen and hydrogen microbubbles are generated at the anode and the cathode, respectively. Then, the metal ions neutralize the charges of the microalgae, larger flocs are formed, and flocculated particles float to the surface with the microbubbles (Emamjomeh & Sivakumar 2009; Gao et al. 2010).

The electrolytic process used in this work was based on the generation of a uniformly varied electromagnetic field by alternate current, comprising the part of the electromagnetic spectrum between the infrared and microwave regions (ranging from 0 to 6,000 cm\(^{-1}\)). According to Lampman et al. (2009), radiation in this energy range includes vibrational frequencies of stretching and bending in most molecules that have covalent bonds and dipole moments, such as water molecules, which have normal symmetric stretch, asymmetric stretch, and folding vibration modes, with wave numbers of 1,643.5 cm\(^{-1}\), 2,127.5 cm\(^{-1}\) and 3,404.0 cm\(^{-1}\), respectively.

In this work, we utilized electroflotation by alternating current (EFAC) with non-consumable electrodes as a method for simultaneous microalgal harvesting and cell disruption from mixed microalgae obtained from waste stabilization ponds.

**MATERIAL AND METHODS**

**Wastewater source and reactor set-up**

Wastewater was collected from a waste stabilization pond system composed of a mechanically aerated facultative pond, a secondary facultative pond, and two maturation ponds in series. The wastewater treatment plant was located in Fortaleza, Ceará, Brazil. Samples were collected from the last maturation pond, near the outlet.

The experiments were performed in two electrolytic batch reactors at laboratory scale. The first reactor (R1) was acrylic, could contain 2.9 L, and had one set of non-consumable electrodes (Figure 1(a)), while the second reactor (R2) was fiberglass, could contain 40 L, and had three sets of non-consumable electrodes (Figure 1(b)). All sets of electrodes were located at the bottom of the reactors (Figure 1(a) and (b)).

The cathode and anode of each set of non-consumable electrodes were made of five steel plates measuring 15 cm \(\times\) 5 cm, with a thickness of 0.2 mm, and spaced 5 mm apart. An external alternating current source was used (HY 125 Hobby, Hayama), applying a voltage of 12 V and a maximum current of 5 A.

**Effect of different frequency ranges on harvesting and microalgal disruption**

In this study, we sought to achieve the superposition principle. In this principle, when two or more waves occupy a certain space at the same time, the displacements caused by each of them are added at each point. Thus, when the crest of a wave is superimposed on another, their individual effects are added and produce a greater wave amplitude. The elevation of this amplitude was reported to disrupt the covalent bonds of water molecules (Lampman et al. 2009), a mechanism different from conventional electrolysis. Bubbles of hydrogen and oxygen gases generated from water electrolysis are essential to harvesting by flotation, as well as the formation of other gases such as nitrogen gas. The fragments H\(^+\) and O\(^2-\) are very reactive and...
promote the formation of oxidant species (O3, H2O2, and –OH), which may effectively act on cell disruption.

In order to verify if cell disruption was caused by the formation of oxidative species or by the frequency variation, an experiment was conducted testing different frequency ranges. The system capacity for both harvesting and disruption of microalgae was evaluated. The batch time was fixed at 20 min for reactor R1, and four ranges of frequencies were tested: 0–1.56, 0–1.78, 0–30, and 0–50 KHz.

Effect of different batch times on harvesting and microalgal disruption

Reactor R2 was operated in batch mode, with a maximum frequency of 1.56 KHz. Samples were taken after 40, 70, 120 and 140 min of batch time. At the end of each batch, a liquid sample of approximately 500 mL was collected from the effluent and analyzed for turbidity in a portable turbidimeter (HACH 2100P), and chlorophyll-a, according to Standard Methods for the Examination of Water and Wastewater (2005).

Lipid extraction

For total lipid yield analysis, all floating biomass was collected manually and stored in 50 mL falcon tubes for subsequent biomass drying by lyophilization (Liotop, L202, Brazil). The methodology of Bligh & Dyer (1959) was applied to 0.5 g of dry biomass in order to extract total lipids. All samples were analyzed in triplicate. The lipid yield was determined by the ratio of the lipid fraction to the initial biomass weight.

RESULTS AND DISCUSSION

Effect of different frequency ranges on harvesting and microalgal disruption

The effects of different frequency ranges on the harvesting capacity were analyzed using turbidity and chlorophyll-a removals (Figure 2). Chlorophyll-a is a well-known parameter that directly measures the presence of microalgal cells (Chen et al. 2011).

The initial turbidity and chlorophyll-a content were 157 NTU and 358 mg/m3, respectively. For all frequency ranges, the observed differences in performance were considered insignificant, with removals of about 80 and 95% for turbidity and chlorophyll-a, respectively. Among frequency ranges tested, there were no observed differences in the system capacity to split the water molecules, which resulted in similar removal efficiencies.

Uduman et al. (2011) achieved algal biomass harvesting at similar rates (about 98 and 99%) for pure cultures of Chlorococcum sp. and Tetraselmis sp. by applying electrocoagulation for 20 min at 10 V, similar to the conditions employed in this work. In other work, Azarian et al. (2007) performed experiments of algal biomass harvested by electrocoagulation by varying the electrical power system (6–550 W/dm3) with a retention time of 10 min. The majority of the chlorophyll-a was removed with a low power of 31 W/dm3 (approximately 70%), and a power of 550 W/dm3 was required to remove 100% of the chlorophyll-a. The authors concluded that a power of 100 W/dm3 required 30 min to complete clearance of the parameter, while for powers of 375 and 500 W/dm3, only 15 min were necessary.

However, in this work, with non-consumable electrodes, efficiencies higher than 80% removal of chlorophyll-a and turbidity could be achieved with a power of 20.7 W/dm3 for 20 min. Furthermore, the methodology used here promoted harvesting without the release of metals into the environment or the need for coagulant chemicals, which reduced process costs.

The effect of different frequency ranges on lipid yield is shown in Figure 3. The results also showed that the
frequency range had no influence on lipid yields. The proposed system was indeed capable of releasing lipids from the microalgal cells, which accumulated on the floating biomass. The capacity increased about 2.5-fold compared with the control, which received no pretreatment. According to Oncel (2013), lipid yield is an indicator of algal biomass potential for biodiesel production.

Koberg et al. (2013) compared the efficiencies of microwaving and ultrasonication for lipid extraction from Nannochloropsis, using an ultrasonicator with a frequency of 20 KHz for 5 min and a microwave oven operating at 2.45 GHz for 5 min at 70% power for a cycle mode of 21 seconds on and 9 seconds off. The authors performed two separate extraction and transesterification steps and reported lipid yields for biodiesel of 18.9% and 32.8% for groups treated with ultrasound and microwave radiation, respectively.

The effect of different batch times on harvesting and microalgal disruption

The effect of different batch times on chlorophyll-a and turbidity removals is shown in Figure 4. Longer batch times led to higher completions of chlorophyll-a removal. However, after a batch time of 70 min, there was a negligible improvement. For the 140 min batch time, the chlorophyll-a removal reached 99% efficiency.

For turbidity removal, there was no significant impact with different batch times, indicating that short batch times could be used. For instance, the removal efficiency was 88% for a 40 min batch time and reached 95% in 140 min.

At 70 min, the turbidity and chlorophyll-a removals were 91% and 93%, respectively. These results are similar to those obtained in reactor R1 for some identical operating conditions (frequency range, set of electrodes, surface area, and current density), indicating that the technology is reliable, and the reactor shape and volume had no influence in the EFAC process.

Poelman et al. (1997) also studied an electrolytic system for microalgal recovery at a laboratory scale. The experiments were conducted in a 100 L reactor volume for up to 75 min of operation with vertical aluminium electrodes. Chlorophyll-a removal was the parameter used to evaluate the harvesting efficiency of microalgae. The authors concluded that electroflocculation was capable of removing, in the first 55 min of system operation, 80–95% of the chlorophyll-a, proving to be an efficient technique. In this work, we found similar results: in 40 min, 75% of chlorophyll-a was removed.

The effect of different batch times on the lipid yield from mixed microalgae is shown in Figure 5. Higher batch times resulted in higher lipid yields, indicating that the proposed system could disrupt microalgal cells and recover the lipids from the floating biomass. For example, the lipid yield increased from about 9% to 14% at 40 min and 140 min batch times, respectively.
Our results agree with those of Wahlen et al. (2014), who evaluated ultrasound as pretreatment (30 seconds) and obtained a 14.4% microalgal lipid yield. They collected mixed microalgae from a stabilization pond, and chloroform and methanol at a ratio of 2:1 (v/v) was added to the samples for lipid extraction. The lipid yield was analyzed after centrifugation.

Lee et al. (2010) compared five methods of cell disruption in pure cultures: (1) autoclaving at 125 °C with 1.5 MPa pressure for 5 min; (2) ball mills, using a ball agitator at 2,800 rpm for 5 min; (3) microwave at 100 °C and 2,450 MHz for 5 min; (4) ultrasound, using a sonicator with a frequency of 10 KHz for 5 min; and (5) osmotic shock by a 10% solution of NaCl stirred for 1 min and allowed to sit for 48 hours. The efficiency of cell disruption for each method was measured by lipid yield, using an extraction with chloroform and methanol 1:1 (v/v). The best results for Chlorella vulgaris cell disruption were achieved with autoclave and microwave pretreatments, which provided lipid yields of about 10%. In the same investigation, microwaving was the best pretreatment for Scenedesmus sp. microalgal cultures, which provided a 12% lipid yield. Using the EFAC process, we found similar and better results, while also promoting microalgal harvesting and cell disruption simultaneously.

Proposed mechanisms for simultaneous microalgal harvesting and cell disruption

A new experiment was conducted to evaluate the presence of hydrogen generated during the electrolytic process. After a 15 min batch time, there was an increase in the gas pressure in a glass flask used, generating a pressure of 70 kPa. The gas composition was 36.2% hydrogen (an average of two batches). This suggests that hydrogen was indeed an important gas used for microalgal harvesting, and the observed pressure increase proved that successful water splitting occurred during the EFAC process. However, other gases may also be important in this process such as Cl2, N2, O2, and O3, which will depend on the wastewater composition and batch time.

CONCLUSIONS

The results revealed that the frequency ranges tested have no influence on turbidity and chlorophyll-a removal. However, high turbidity and chlorophyll-a removal was achieved even with a short batch time.

The proposed system was capable of releasing lipids from the mixed microalgae, showing an increase of about 2.5-fold compared with the control, a non-conventional electroflotation application.

The batch time positively correlated with both clarification capacity and cell disruption, but it is possible that a short retention time, for instance 70 min, could be used.

To the best of our knowledge, this is the first attempt at using EFAC for simultaneous microalgal harvesting and cell disruption, with promising results not only for open systems, such as stabilization ponds, but also for closed systems such as photobioreactors.

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