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Flocculation of *Arthrospira maxima* for improved harvesting

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Abstract

The environmental impacts associated with the burning of fossil fuels coupled with growing concerns about security of energy supply, motivated the search for more sustainable forms of energy production, among which came microalgae for biofuels production. However, the commercial production of microalgae biofuels is still not competitive compared to fossil fuels, as it is necessary to solve some process bottlenecks, among which biomass harvesting, that is the focus of this work. Hence, this work intends to study the harvesting of microalga *Arthrospira maxima* through flocculation by pH variation and/or addition of $CaCl_2$ as flocculant. Thus, it is described the effect of pH variation (in the range 6 to 12), followed by the addition of flocculant, on the harvesting efficiency. Results show that by pH increase over 10 using NaOH, or by flocculation using $CaCl_2$ at a concentration of 0.2-2.0 g/L and at a 1:30 ratio (v/v) of $CaCl_2$/microalgae culture, it is possible to effectively harvest this microalga.

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Keywords: Arthrospira maxima; Coagulation; Flocculation; Microalgae harvesting

1. Introduction

Biofuels are a renewable energy source that can be used as an alternative to fossil fuels, with environmental advantages, such as lower CO_2 emissions and lower impacts on resources depletion and greenhouse gas emission [1]. However, first generation biofuels are produced from traditional food or feed crops, raising serious social and environmental issues [2]. Thus, the search for alternative potential feedstocks for biofuels production, has driven researchers' attention to unicellular microorganisms such as microalgae [3]. Microalgae cultivation does not need large footprint area, nor arable land, when compared to traditional food crops. Also, microalgae show high growth rate and contain higher lipid content than the traditional oil seeds [4].

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Among microalgae, this study chose *Arthrospira maxima* (*A. maxima*), a free-floating filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open left-hand helix. These photosynthetic organisms, also considered as blue–green microalgae, occur naturally in tropical and subtropical lakes with alkaline pH and high concentrations of carbonate and bicarbonate [5]. Because *Arthrospira* is very rich in proteins (53 to 68% by dry weight) it is widely used as a food supplement for humans and animals. In addition, it contains high amounts of polyunsaturated fatty acids (PUFAs), about 1.5–2 percent of the total lipid content of 5–7% [6].

Production of microalgae biomass includes microalgae cultivation on environmental conditions that favor accumulation of target metabolites and recovery of the microalgal biomass for downstream processing [7]. The large scale harvesting of microalgal biomass from the culture medium still poses challenges, due to their reduced cells size (2–50 *m*m), the negative surface charge (of about $-7.5 \sim -40$ mV) and the relatively low biomass concentration (0.5–5 g L^{-1}), representing more than 30% of the total production cost of microalgae biodiesel [8].

The main factors for efficient harvesting and for making profitable the production of microalgae biofuels are the reduction of energy consumption [9] and the possibility of nutrients recycling and the reuse of the culture medium [10]. Therefore, it is required the development of cost-effective and more sustainable technologies for microalgae harvesting [11]. In this regard, flocculation can be a method with lower energy consumption when compared to filtration or centrifugation that makes it more feasible economically [12]. In addition, flocculation allows the harvesting of large amounts of biomass without disruption of the cells, thus becoming a more reliable and high-yielding process. A way to induce flocculation without the use of a flocculating agent is through pH variation in the microalgae cultures [13,14], where flocculation at high pH is normally due to precipitation of $CaCO_3$, calcium phosphate and $Mg(OH)_2$ [15,16]. However, depending on algal species being cultured the pH threshold for flocculation vary [17].

A wide variety of flocculants, such as organic cationic polymers or inorganic metal ions, have been exploited for algae harvesting [18–20]. However, the flocculants efficiency mainly depends on the microalgae characteristics, such as cell wall composition, cells size, culture age and growth medium composition and pH [21,22]. The large-scale harvesting using flocculants depends on the microalgae species, the amount of flocculant required and the pH at which the flocculant will have the desired efficiency [23]. For example, Bracharz et al. [24] employed auto-flocculation and biological flocculants to harvest *S. obtusiusculus* from a cultivation media containing high salt concentrations, studying flocculation induced by pH-shift and by addition of two biological flocculants, chitosan and the commercial tannin CFL-PT. These authors concluded that in comparison to biological flocculants, induction by pH shift is cheaper, but due to buffering effects of the brackish cultivation medium infeasible amounts of base are required to raise the pH-value. On the other hand, tannin seems to be superior compared to chitosan. Pandey et al. [25] performed an experimental study to evaluate the comparative efficiency of a bio-flocculant (waste egg shell), calcium carbonate and $Al_2(SO_4)_3$ for harvesting *Chlorella pyrenoidosa* and studied the influence of pH on zeta potential to explain the chemistry of flocculation process. Chen et al. [26] used ammonia as microalgae flocculant of both freshwater (*Chlorella sorokiniana*) and marine algae (*Nannochlropsis oculata*), with the advantage of the possibility to reuse the ammonia added as fertilizer in the subsequent cultures, and showing more than 99% algae removal at 12 h with this approach. Augustine et al. [23] evaluated the flocculation processes using chemo-magnetic nanoparticle (Che-MNP) and chitosan magnetic nanoparticle (CS-MNP) composites, showing that these allow efficient harvesting, being possible to reuse the flocculant (MNP) and flocculated medium without affecting the integrity of the algae cells.

Wang et al. [27] compared centrifugation of microalgae *Scenedesmus obliquus*, as a standard harvesting method, with chemical flocculation, as a cost-effective harvesting method. Results showed that lipid recovery from the centrifuged cells was 17.4%, which significantly increased by flocculation to 20.7%. Both harvesting methods presented similar thermal decomposition patterns, but the flocculated biomass showed a 15.7% higher bio-char formation than the centrifuged cells, which resulted in significant reduction in the bio-oil yield by 18.5%.

Vandamme et al. [28] studied the effect of the harvesting method on the lipid content and the lipid extraction efficiency of *Phaeodactylum tricornutum* biomass, comparing alum or alkaline flocculation, and centrifugation, showing that alkaline flocculation can be an excellent primary harvesting method for this microalga without impacting its lipid extraction efficiency. Therefore, this research aims to compare and evaluate the effectiveness of auto-flocculation and flocculation approaches applied to *Arthrospira maxima* harvesting, induced by pH variation and by the addition of $CaCl_2$ as flocculant.

2. Materials and methods

Arthrospira maxima SAG 84.79 from the German SAG (Sammlung von Algenkulturen Göttingen) collection was used in this work, following different cultivation scales, from 20 mL tubes to 60 L tanks. Among the several chemicals referred in literature, as potential coagulation/ flocculation agents, $CaCl_2$ was used and tested in this study at different concentrations.

The effect of pH variation on coagulation/flocculation of *Arthrospira maxima* was analyzed, for which the purpose of pH increase and decrease was achieved respectively by adding NaOH or HCl. Cultures with an initial pH of 10 were poured into 250 ml beakers, and the pH was adjusted to 9, 8, 7 and 6 in the culture medium. Samples were taken at 15 min intervals and absorbance was measured at 680 nm, for a maximum 4 h of settling time.

The biomass removal efficiency was calculated for each sample. Similar procedure was followed by adding NaOH to increase the culture medium pH to 11 and 12.

The effectiveness of $CaCl_2$ as flocculant on coagulation and flocculation was evaluated: after adding the chemical compound, the microalgae culture was stirred for 1 min at 75 rpm followed by 15 min stirring at 25 rpm, and then settling, in a jar test apparatus. Aliquots of the clarified liquid were taken for absorbance measurement (at 680 nm) at different time intervals until 4 h of settling time.

3. Results and discussion

The culture medium pH was increased from 10 to 11 and to 12 using NaOH, resulting in coagulation after 15 min. The coagulated cells remained suspended in the growth medium (as shown in Fig. 1).

Furthermore, by adding $CaCl_2$ as flocculant, testing concentrations from 0.2 to 2.0 g/L of 10 ml solution in 300 ml of microalgae culture, the flocculation occurred after 15 min. Similarly, the microalgae flocculated in the bottom of the recipient.

Therefore, results showed that *Arthrospira maxima* can be effectively harvested by increasing the pH over 10 with NaOH or by adding $CaCl_2$ as flocculant with a concentration of about 0.2–0.3 g/L or a 1:30 volume ratio of $CaCl_2$/microalgae culture, respectively. These chemicals are easy to use, not expensive, and do not pose any substantial contamination risks for the recovered microalgae biomass for further processing. Other advantages include the operational simplicity and the possibility of the culture medium to be reuse and the nutrients recycled.

Literature studies on flocculation with pH variation reported similar results. For example, Sukenik and Shelef [15] reported algae auto-flocculation in outdoor autotrophic cultures of *Scenedesmus dimorphus* when culture pH was raised due to photosynthesis. Kim et al. [29] induced auto-flocculation of *Arthrospira platensis* at an optimal pH level of 9 with a flocculation efficiency of almost 90%, showing that this approach is definitely useful to harvest these microalgae in large-scale culture without any drawbacks of harvested algal biomass. Liu et al. [30] reported that metal ions, such as Mg^{2+} and Ca^{2+}, in the growth medium are hydrolyzed to form positive precipitates, which coagulate negative microalgae cells by sweeping flocculation and charge neutralization. In addition, flocculation efficiency is induced by pH increase, but it considerably decreases with increase of biomass concentrations. Vandamme et al. [31] demonstrated that *Chlorella vulgaris* flocculation can be induced by increasing medium pH to 11 and that magnesium is essential to induce flocculation. Yahi et al. [32] reported that extensive algae flocculation can be induced at pH values between 11.8 and 12 without need of adding magnesium.

Wu et al. [33] showed that by increasing the medium pH, a flocculation efficiency of up to 90% was induced for freshwater microalgae (*Chlorella vulgaris, Scenedesmus sp., Chlorococcum sp.*) with low/medium biomass concentrations and marine microalgae (*Nannochloropsis oculata, Phaeodactylum tricornutum*).

Liu et al. [34] developed a flocculation method to harvest target microalgae with self-flocculating microalgae induced by decreasing pH to just below isoelectric point, showing a higher efficiency of this approach when compared to flocculation only via pH decrease. With this method the culture medium could be recycled for cultivation after neutralizing the pH and adding more nutrients.

Spilling et al. [17] studied the effect of pH increase on flocculation of the diatom *Phaeodactylum tricornutum* and of the green algae *Scenedesmus cf. obliquus*, showing that *P. tricornutum* started to flocculate at pH 10.5 and *S. cf. obliquus* at pH 11.3. Also, it was studied the combined effect of pH, turbulence, and cell density on the flocculation of *P. tricornutum*, showing that pH is the most important factor affecting flocculation. These authors also reported that since algae cultures increase the pH during photosynthesis, and *P. tricornutum* and *S. cf. obliquus* cultures increased the pH to a maximum of respectively 10.8 and 9.5, which for *P. tricornutum* is above the

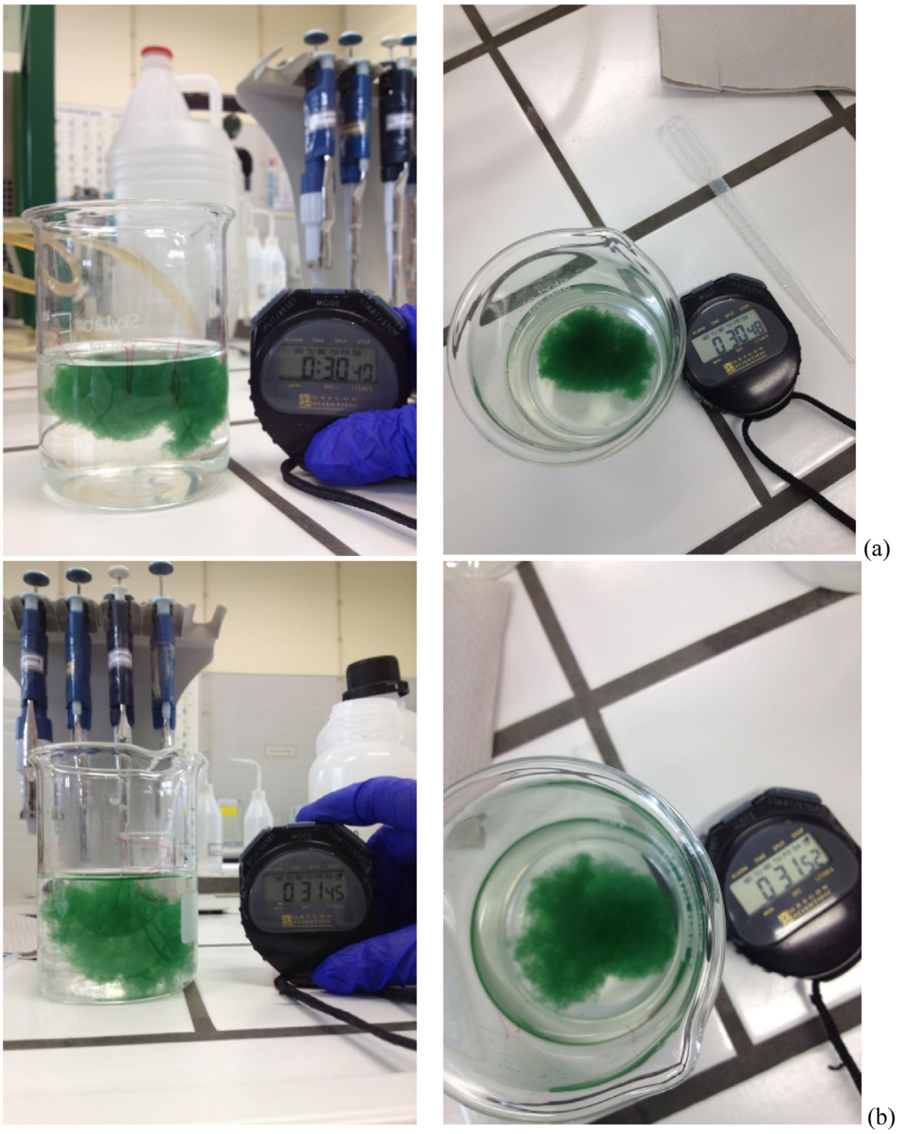

Fig. 1. Evolution of the samples after addition of NaOH, with the culture medium at (a) pH 11 and at (b) pH 12. Photos on the left are the front view and on the right the top view.

flocculation threshold, it was observed a rapid settling of this species in a matter of hours after discontinuing the CO_2 supply. Thus, the authors suggested that this could be used as a simple and low-cost initial dewatering step for *P. tricornutum*.

4. Conclusion

This study compared and evaluated the effectiveness of coagulation–flocculation methods for harvesting *Arthrospira maxima*, induced by pH variation and addition of flocculants. Results showed that, for the range of conditions tested, *Arthrospira maxima* can be effectively harvested by increasing pH over 10 with NaOH or by adding $CaCl_2$ as flocculant, with no need for addition of other chemicals. These chemicals are easy to use, inexpensive, and do not pose any substantial contamination risk of the recovered microalgae biomass. Therefore, this method can contribute to the economical harvesting of microalgae biomass.

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