EVALUATION OF THE EFFECT OF FLOCCULATION ON HARVESTING OF MICROALGAENANNOCHLOROPSIS SP

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ABSTRACT

Microalgae have recently emerged as an attractive source of products for the food, energy and pharmaceutical industries due to their high biomass productivity, metabolic versatility and environmental sustainability. This work focuses on the effect of flocculation on the harvesting of microalgae *Nannochloropsis*sp. Through a three-factor experimental design. The most suitable conditions for flocculation were determined based on flocculant ($AlCl_3$) dosage, pH and culture time. In addition, flocculant adding-pH adjustment methods were studied and harvesting was performed according to the method that provided the best results. The pH adjustment after flocculant addition showed higher flocculation efficiency (above 90%) compared to the reverse process. It was also found that the microalgae cells exhibited spontaneous sedimentation, suggesting that the use of flocculant is not required for biomass collection.

Keywords: microalgae, harvesting, flocculation, sedimentation, pH.

INTRODUCTION

Current industrial society depends on conventional energy sources like coal, natural gas, oil and uranium, which lead to serious environmental problems and depletion of resources(Kalidasan *et al*., 2015).Environmental and economic sustainability issues strongly support the use of renewable resources (El-Mekkawi *et al*., 2016).Microalgae are attracting enormous attention from the scientific community worldwide as renewable feedstock for energy production (Sunar *et al*., 2016). Microalgae are single-celled or colonial photosynthetic organisms that are naturally present in different aquatic/humid environments, including rivers, lakes, oceans and soils (Brasil *et al*., 2016). They have the advantages of higher productivities, compared to oil seeds, no competition with food crops and ability to grow under extreme conditions (Collotta *et al*., 2017). Microalgae can also be regarded as an attractive source for producing valuable bioproducts, such as polyunsaturated fatty acids, carotenoids, phycobiliproteins, polysaccharides and phycotoxin(Sathasivam *et al*., 2017). As energy source, the high yield of biofuel produced from microalgae relies on the large amounts of neutral lipids accumulated in microalgae cells, which can further be increased by acting on growth conditions (Zakariah *et al*., 2017).

Biofuel production from microalgae involves biomass recovery from the culture medium, followed by the extraction of lipids or other components. Many strategies have been proposed for harvesting, including centrifugation(Knuckey *et al*., 2006), filtration(Zhang *et al*., 2010), flotation(Chen, Liu, & Yih-Hsu, 1998) and flocculation(Sari & Purnawan, 2016). Similarly, the recovery of intracellular components by efficient techniques is the subject of intense research (Zuorro *et al*., 2016a and b; Maffei *et al*., 2018).

Flocculation is considered the most suitable option for concentration of the biomass because it allows the collection of large amounts of biomass at low cost and can be easily scale scaled(Borges *et al*., 2011). The mechanism of flocculation involves charge neutralization, in which positively charged flocculants are attracted to the negatively charged colloids via electrostatic interaction, followed by the formation and growth of flocs and final agglomeration (Kim *et al*., 2017).

Flocculants are classified into inorganic flocculants, as polyaluminum chloride (PAC), synthetic organic flocculants, as polyacrylamide derivatives, biomaterials, as chitosan, and nanoparticles such as nanomagnetite(Sari & Purnawan, 2016). For more practical applications, flocculation using metallic coagulant such as aluminum and ferric salts, commonly known as inorganic chemical flocculation, is considered the most promising method because of its simplicity and low energy requirement (Kim *et al.,* 2017). It has been reported that flocculation by metallic salts can be carried out under acidic or alkaline conditions (Garzón-Sanabria *et al*., 2012). According to Uduman *et al*. (2010), metallic salts of iron or aluminum are preferred for acidic pH conditions. In addition, the effectiveness of sedimentation with this type of flocculant depends on the initial biomass concentration and dose (Garzón-Sanabria *et al*., 2012).Rwehumbiza *et al*.(2012) evaluated the effects of aluminum as flocculant on the extraction of lipids and methyl esters of fatty acids for producing biodiesel from *Nannochloropsis*sp., obtaining efficiencies between 79 and 99%. After extraction and conversion of lipids to biodiesel, with an initial biomass concentration of 20 g/L, it was found that aluminum was not detectable and at lower initial biomass concentration sit decreased two to three times.

In order to provide further insight into the role and impact of flocculation on microalgae processing, this work investigated the effects of flocculation with metallic $AlCl_3$ on biomass collection from *Nannochloropsis* sp.

METHODOLOGY

Culture methods

*Nannochloropsis*sp. was isolated from Gulf of Morrosquillo (Sucre-Córdoba, Colombia), which was cultivated in Bold Basal medium. Main components of this medium are (mg/L): $NaNO_3$ (2.94), $MgSO_4 \cdot 7H_2O$ (3.04×10^{-1}) NaCl (4.28×10^{-1}), K_2HPO_4 (4.31×10^{-1}), KH_2PO_4 (1.29), $CaCl_2 \cdot 2H_2O$ (1.70×10^{-1}), H_3BO_3 (1.85×10^{-1}), EDTA (1.71×10^{-1}), KOH (5.53×10^{-1}) and $FeSO_4 \cdot 7H_2O$ (1.79×10^{-2}). The microalgae were kept growing in 12 L glass reactor equipped with a bubble aeration system and light-dark cycle 12:12 h.

Experimental design

Experimental design was performed using STATISTICA 7.0 program based on the procedure described by Garzón-Sanabria *et al.* (2012). Factors considered in this study are shown in Table-1: culture time (5, 10 and 15 days), pH (4, 7 and 10) and concentration of flocculant (50, 100 and 150 mg/L).

Table-1. Experimental design layout.

Test	**Culture time** (days)	**Dosage of flocculant** (mg/L)	pH
1	1.63	100	7
2	5	50	4
3	5	50	10
4	5	150	4
5	5	150	10
6	10	16.3	7
7	10	100	1.9
8	10	100	7
9	10	100	7
10	10	100	7
11	10	100	12
12	10	183.6	7
13	15	50	4
14	15	50	10
15	15	100	4
16	15	100	10
17	18.3	100	7

Determination of optimal conditions for flocculant adding-pH adjustment

Preliminary experiments were made to evaluate the optimal flocculation procedure (pH adjustment before or after adding flocculant). A solution of 40 g/L of flocculant agent ($AlCl_3$) was prepared and then diluted to obtain aliquots of 50, 100 and 150 mg/L, which were added to 100 mL of sample. The pH adjustment was performed with 1M HCl and 1 M NaOH to get values of 4, 7 and 10. Samples were stirred at 500 rpm for 2 min, followed by slower mixing at 60 rpm for 15 min in order to allow the formation of aggregate and hydrolysis of flocculant. Finally, the pH was measured and samples of 10 mL were collected at 1-h intervals during 5 h to quantify the optical density of *Nannochloropsis* sp. at 750 nm.

Flocculation tests

Microalgae were cultivated with an inoculum of 2.5 g/L of *Nannochloropsis* sp. wet biomass. Tests were carried out as shown in Figure-1. An optimal method was used to determine the initial and final absorbances, with the efficiency calculated according to Equation-1.

$$\text{Efficiency (\%)} = \left(1 - \frac{\text{final absorbance}}{\text{initial absorbance}}\right) \times 100 \qquad (1)$$

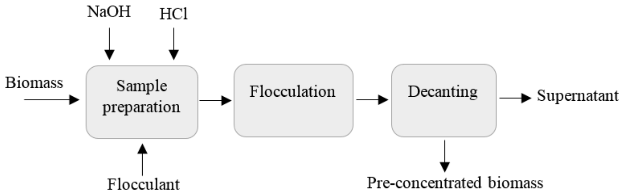


Figure-1. Experimental procedure.

RESULTS

Addition of $AlCl_3$-pH adjustment

Figure-2 shows the trends of pH with time for evaluating the best conditions for flocculant addition and pH adjustment with the two methods previously described. As can be seen, the pH remained approximately constant when flocculation occurred; therefore, the method chosen for developing the experimental design was the one providing a higher efficiency.

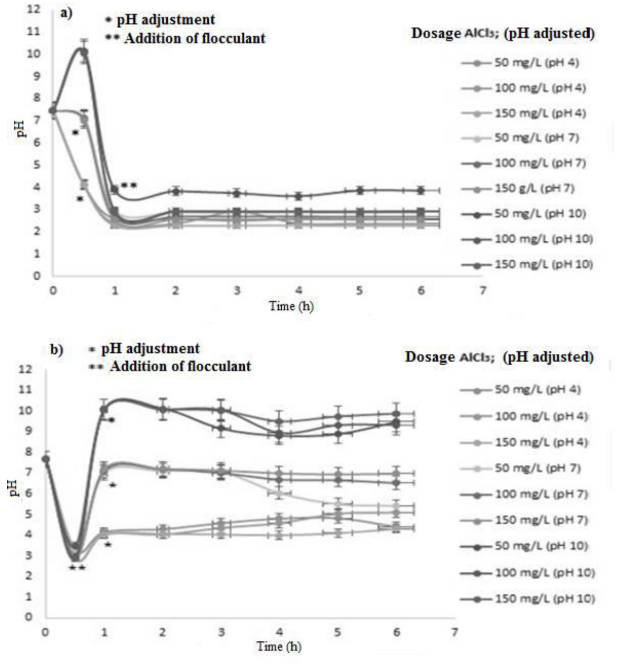


Figure-2.pH behaviour as a function of time: pH adjustment before (a) and after (b) addition of $AlCl_3$.

Figure-3 shows that the flocculation efficiency was higher (values above 90%)when adjusting the pH after addition of flocculant. Other studies have also reported high efficiencies for harvesting *Nannochloropsis* sp. with different flocculants as summarized in Table-2. The maximum flocculation efficiency obtained in this work is similar to that reported by Garzon-Sanabria *et al.*(2013), who also used an aluminum salt at different flocculant dosages.The optimal flocculant dosage for achieving the highest efficiency on removal of the microalgal cells from the culture was 100 mg/L. The variations of efficiency with flocculant dosage are due to the microalgal cell surface charge, which affects the stability of the cell suspension. Cationic flocculants such as aluminum salts neutralize the surface charge on the cells to facilitate the formation of flocs; hence, the dosage of flocculant required to produce effective flocculation depends on surface charge(Chatsungnoen& Chisti, 2016).

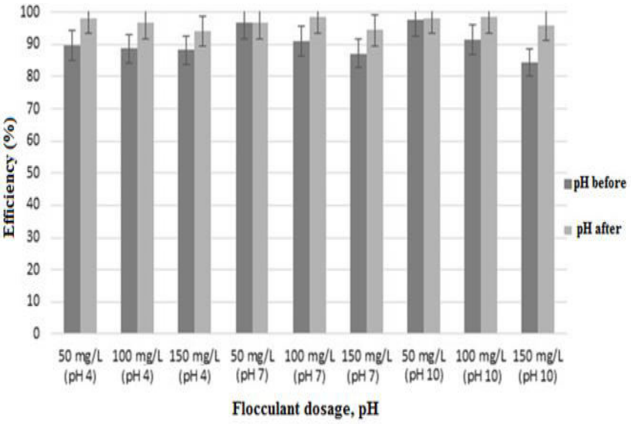


Figure-3. Comparison of flocculation efficiency for two methods of flocculant addition and pH adjustment.

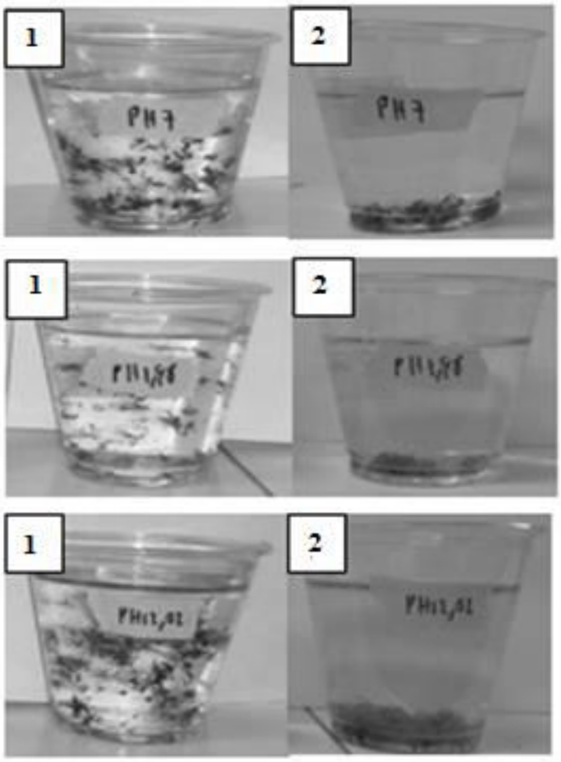
Table-2. Summary of different flocculants successfully used for harvesting *Nannochloropsis* sp.

Flocculant	Maximum Recovery (%)	Flocculant dosage	Reference
Chitosan	90	100 mg/L	(Sadegh *et al.*, 2013)
Tanfloc	98	10 mg/L	(Roselet *et al.*, 2016)
$AlCl_3$	98	50 mg/L	(Garzon-Sanabria *et al.*, 2013)
Bioflocculant from *Solibacillussilvestris*	90	0.1 % (w/v)	(Wan *et al.*, 2013)
$AlCl_3$	97	100 mg/L	This work

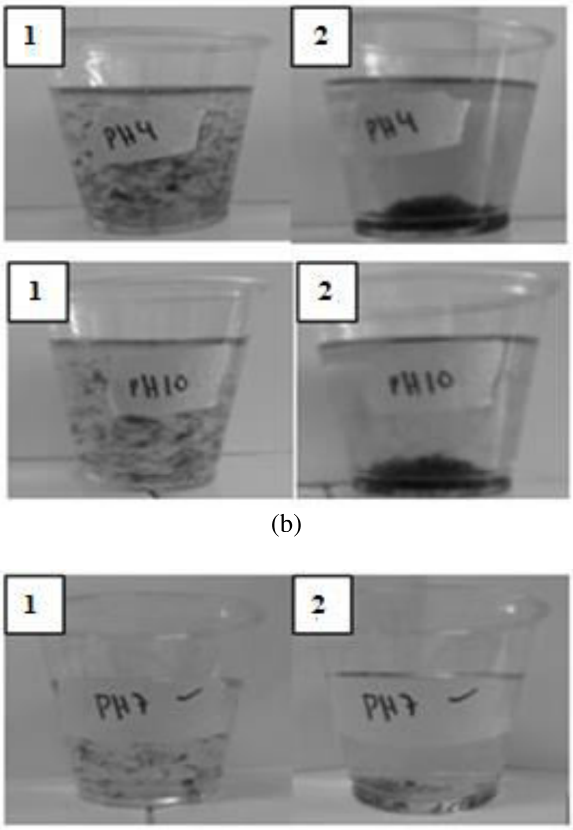
Flocculation tests

According to the tests carried out for experimental design with *Nannochloropsis* sp., spontaneous flocculation occurred in all the experiments. For samples corresponding to 1.6 and 5 days, an attempt was made to measure the flocculation by spectrophotometry, however, this procedure was not possible to perform because cells already had agglomerated before starting samples preparation since precipitation occurred immediately. For 10, 15 and 18.3 days, tests were carried out according to experimental design and without flocculant as control; all the tests were photographed (not shown). The recording of control samples (Figure-4) shows that incorporating flocculant was not necessary and pH did not have a relevant influence on sedimentation process, since the precipitation was the same in all experiments.

It is evident that the performance of the biomass used for experimental design and for best flocculant adding-pH adjustment method is different. The growth was different for both strains, since in the first there was the formation of aggregates facilitating the sedimentation; this is because the cultivation of this strain was carried out in fresh water and not in marine water, where this species of microalga usually grows.



(a)



(b)

(c)

Figure-4. Control samples for the experimental design. (a) 10 days of culture; (b) 15 days of culture; (c) 18.3 days of culture. (1) Freshly stirred sample; (2)Samples after some seconds of agitation.

CONCLUSIONS

This work investigated the effects of flocculation on harvesting *Nannochloropsis* sp. High flocculation efficiencies (above 90%) were obtained, suggesting that flocculation is a suitable method for biomass recovery from the culture medium. However,microalgae culture exhibited spontaneous sedimentation; hence, the use of flocculant was not required for biomass collection. In addition, pH adjustment after adding flocculant indicated higher flocculation efficiencies compared to the reverse method.

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