CHANGE OF CULTURE BROTH pH FOR MICROALGAE SEPARATION FROM THE GROWTH SOLUTION

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Abstract. *The separation of the microalgae from the growth solution is an important procedure before the drying and oil extraction from the biomass. Previously published researches showed that pH increase of Dunaliella tertiolecta culture broth causes flocculation of the microalgae, probably because it reduces the electrical charge on the surface of algal cells which works as a fundamental force to stabilize the suspended particles. The purpose of this study is to demonstrate the efficiency of the increase in pH of a Nannochloropsis oculata microalgae solution, by sodium hydroxide (NaOH) addition, to the flocculation, making possible a simple harvesting of the cells. The microalgae were grown in 0.2 m³ tanks for 10 days in a greenhouse under aeration. After this time, with a considerable cell concentration at the culture broth, a 1.0 M (molar) sodium hydroxide solution was added, increasing the pH. The system was kept under agitation during the addition by an air pump into the tank. Thereafter the system was maintained at rest. After a short time, it was observed that the microalgae coagulated and settled. The upper clarified water was removed, remaining a concentrated microalgae solution which will be sent to drying, lipid extraction and biodiesel synthesis. Our results suggest that pH increase of the culture broth is a suitable methodology for microalgae separation from the growth solution. Similar results were obtained with cultures of Phaeodactylum tricornutum.*

Keywords*: pH increase, microalgae flocculation, biodiesel synthesis, Nannochloropsis oculata, Phaeodactylum tricornutum*

1. INTRODUCTION

Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Like plants, microalgae use sunlight to produce oils but they do so more efficiently than crop plants. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops (Chisti, 2007).

There are a lot of possibilities of microalgae cultivation for biodiesel production, but all options are limited to aqueous environments. These microorganisms grow in appropriate culture broth, consisting basically by water, minerals and vitamins. So, is necessary to separate the microalgae to sequence de biodiesel production.

The separation of microalgae from the culture broth is an important unit operation at the biodiesel production from this source. To have as a feature a small constitution, it's hard to do this separation. Gwo *et al.* (2005) characterized *Nannochloropsis oculata* like green microalgae, with spherical or ovoid cells, and 2 to 5 μm in diameter. For this scale, the separation of the algae by filtration becomes a difficult process, because of the small porosity required. The use of special membranes would an expensive alternative, which it's not practicable in large production scale.

Flocculation is a universal process occurring within aquatic ecosystems that incorporate both inorganic and organic cohesive particles (Droppo *et al.*, 2005). This method is commonly used in water and wastewater treatment, with the objective to remove contaminants, like microalgae, by decantation. To flocculate, flocculating agents are used, which, in contact with the solution, cause the formation of particle clusters, facilitating the settlement.

Horiuchi *et al.* (2002) demonstrate that pH increase of the culture broth using a sodium hydroxide (NaOH) solution, caused the flocculation and settlement of *Dunaliella tertiolecta* microalgae, resulting in a recuperation of 90 % of the solution cells.

The flocculation process has as foundation the reduction of the electrical charge on the surface of algal cells. This charge, known as ξ-potential, works as a fundamental force to stabilize the particles while in suspension. Thus, we suppose that the pH change of the growth solution of microalgae of interest is an effective way to separate this one of the culture broth.

This paper reviews the experiments of *Nannochloropsis oculata* microalgae flocculation by pH change of the culture broth, seeking the validation of this hypothesis to that microorganism, also find the best pH value to faster decantation.

2. MATERIAL AND METHODS

The microalgae growth was made during 10 days, in three 0.2 m^3 tanks, containing appropriate culture broth, in a greenhouse, and under aeration. This task was coordinated by Grupo Integrado de Aquicultura e Estudos Ambientais from Federal University of Paraná.

After the cultivation get a good cellular concentration, the flocculation by pH change tests were made. A 1.0 M (molar) sodium hydroxide (NaOH) solution was prepared. The tanks were maintained under agitation using an air pump positioned at their bottoms. The temperature was measured using a thermometer present in a digital oximeter. Was added to the tanks, slowly, the NaOH solution, controlling the pH with a digital pHmeter, as shown in Fig. 1.

Figure 1. Equipment for pH measurement.

With the pH near 10.0, and occurring the flocculation, were defined three different final volumes of NaOH solution to be added in the tanks, as shown in Tab. 1. The final pH and temperatures of the tanks were measured. The air pumps, which provide the agitation, were turned off, and the settlement time was registered. The final time was established maintaining the same visual standard in the three tanks.

Table 1. Final 1.0 M NaOH volumes added to the tanks.

Experiment	NaOH volume (m^3)
TQ-1	8.0×10^{-4}
TQ-2	6.0×10^{-4}
TQ-3	8.5×10^{-4}

After the settlement in the three tanks, the supernatant was separate from the flocculated material, which was packed in 0.02 m^3 bottles, as shown in Fig. 2.

Figure 2. Flocculated microalgae.

For qualitative information, the same procedure was made in a 0.2 m^3 tank with *Phaeodactylum tricornutum* microalgae, adding $8.5x10^{-4}$ m^3 of 1.0 M NaOH solution.

Aiming to observe the influence of the temperature at the flocculation and settlement process, three gallons with $1.4x10^{-2}$ m^3 of growth solution, containing *Nannochloropsis oculata* microalgae, were placed under heating, using resistance heaters, connected to thermostats, as shown in Fig. 3. These gallons were kept under agitation using air pumps, positioned at their bottoms. Table 2 shows the experiments names and respective tested temperatures.

Table 2. Applied temperatures in $1.4x10^{-2}$ m^3 experiments.

Experiment	Temperature (K)
GL-1	299.95
GL-2	303.05
GL-3	300.95

Figure 3. Experiment of temperature change.

To each gallon was added $5.0x10^{-5}$ m^3 of 1.0 M NaOH solution, causing microalgae flocculation, and after settlement. The pH values were measured before and after sodium hydroxide addition, and the time to settlement was registered.

3. RESULTS AND DISCUSSION

The experiments results are summarized in Tab. 3 to Tab. 5, for pH change experiments in 0.2 m^3 tanks, and for temperature change, with pH change, in $1.4x10^{-2}$ m^3 gallons, both with *Nannochloropsis oculata* solutions.

Table 3. Data from pH change experiments in 0.2 m^3 tanks, containing culture broth volume values (V_m), and inicial (T_0) and final (T_e) temperatures to NaOH solution addition.

Experiment	V_m (m^3)	T_0 (K)	T_e (K)
TQ-1	0.175	293.25	293.85
TQ-2	0.170	293.05	293.65
TQ-3	0.170	293.05	293.85

Table 4. pH values variation with added volume of 1.0 M sodium hydroxide solution (V_{NaOH}).

Experiment		Experiment		Experiment	
TQ-1		TQ-2		TQ-3	
V_{NaOH} (m^3)	pH	V_{NaOH} (m^3)	pH	V_{NaOH} (m^3)	pH
0	8.73	0	8.87	0	8.85
$2.0x10^{-4}$	9.85	$2.0x10^{-4}$	9.89	$2.0x10^{-4}$	9.89
$3.0 x10^{-4}$	10.09	$3.0 x10^{-4}$	10.08	$3.0 x10^{-4}$	10.08
$4.0 x10^{-4}$	10.13	$4.0 x10^{-4}$	10.11	$4.0 x10^{-4}$	10.11
$5.0 x10^{-4}$	10.14	$5.0 x10^{-4}$	10.14	$5.0 x10^{-4}$	10.13
$6.0 x10^{-4}$	10.14	$6.0 x10^{-4}$	10.15	$6.0 x10^{-4}$	10.13
$8.0 x10^{-4}$	10.09	-	-	$8.5x10^{-4}$	10.08

In all of the three experiments, was observed start of flocculation with addition of $3.0x10^{-4}$ m³ of NaOH solution, but with little cell clusters, which probably will settle slowly. So, it was added more flocculant solution, causing formation of biggest cell agglomerations. After $6.0x10^{-4}$ m³ solution addition, was observed the formation of big clusters, reason why the experiment TQ-2 was lower number of additions, with volume fixed in $6.0x10^{-4}$ m³. For the other tanks, were added more flocculant solution, aiming to compare the volume and pH influences in settlement velocity.

The values of decantation time for each tank were accompanied, with results shown in Tab. 5. Figure 4 shows three times of the flocculation experiment.

Table 5. Decantation time of flocculated microalgae, for same visual standard of clarified supernatant.

Experiment	Time (s)
TQ-1	» 12000.0
TQ-2	» 12000.0
TQ-3	3000.0

Figure 4. Change of pH experiments tanks before flocculation, during settlement and after settlement.

As shown in Tab. 5, the TQ-3 experiment presented lower decantation time. However the final pH was next of experiment TQ-1, was added greater quantity of NaOH in the third tank. A superior quantity of flocculant caused formation of biggest cells agglomerations, resulting in a best decantation.

In two tanks where added more quantity of flocculant, was observed a decrease in pH value, after the last addition. This reduction was not expected, since that the quantity of OH^- ions in the solution are increased. It is believed that the temperature increases of the broth, because of environmental factors, influenced in this pH measure. The experiment TQ-2 can not be associated in this parallel, because of the pH measure after NaOH addition didn't agree with the final temperature measure, like occurred in the other two experiments.

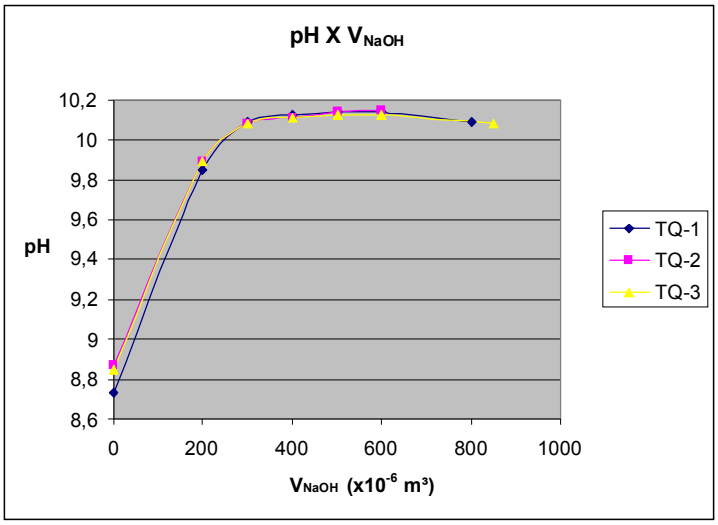

Figure 5. Variation of culture broth pH with addition of 1.0 M sodium hydroxide solution.

Figure 5 shows that after achieved a pH higher than 10, even with basic solution addition, there is a little pH variation. However, as said previously, the increase of NaOH solution volume favored the formation of biggest cells agglomerations, facilitating the settlement.

Horiuchi *et al.* (2002) mentioned, in her work with *Dunaliella tertiolecta* microalgae, that the coagulation mechanism details are unclear at the moment, however, it is speculated that the pH increases may reduce the electrical charge (ζ-potential) on the surface of algal cells, which works as a fundamental force to stabilize the suspended particles.

Even with the pH stabilization, we can suppose that the increase of NaOH solution addition has increased the number of ions in the solution, influencing the cellular charges, and favoring the flocculation. This explains lower decantation time in the tank where was added more sodium hydroxide solution.

Concerning the temperature increases experiments, the addition of 5.0×10^{-5} m³ of 1.0 M sodium hydroxide solution in 1.4×10^{-2} m³ of culture broth was taken as standard for the three tests, obtaining pH and time values exposed in Tab. 6.

Table 6. Values of final pH and time to decantation of temperature change tests.

Experiment	Temperature (K)	Initial pH	Final pH	Decantation time (s)
GL-1	299.95	9.71	9.81	2700.0
GL-2	303.05	9.57	9.70	4500.0
GL-3	300.95	9.67	9.78	3000.0

It can be observed from the temperature change experiments data that same solutions have different initial pH values, because of different temperatures. Lower temperatures imply higher pH values, which caused decrease in decantation time, for providing formation of higher microalgae clusters.

About the tank with *Phaeodactylum tricornutum* microalgae, the settlement occurred faster than *Nannochloropsis oculata* tank, for the same experiment, since the first has higher diameter than the second specie, forming higher clusters. To be only qualitative experiment, the variables were not controlled.

4. CONCLUSIONS

The microalgae flocculation process by change of pH culture broth shown to be an efficient tool for separation of the cells from the growth solution. The living cells are equipped with cilia, and in constant motion, making natural settlement almost impossible. However, the small cellular diameters make the settlement a slow process and, consequently, impracticable in a great production demand, as is the case of microalgae application for biodiesel production. Then, the validation of the hypothesis of sodium hydroxide utilization as *Nannochloropsis oculata* and *Phaeodactylum tricornutum* flocculant was important, since these varieties of microorganisms are fundamental lipid sources in biodiesel from microalgae production systems.

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7. RESPONSIBILITY NOTICE

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