THE EFFECT OF TEMPERATURE IN TETRADESMUS OBLIQUUS

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ABSTRACT

The ever growing demand of energy generation and distribution has been one of the concerns of governments and the focus of research institutions. Likewise, how to supply the energy demands necessary for the development of nations having the lowest environmental impact possible has also been studied. Biofuels have been pointed out as an alternative for that energy challenge, since their use reduce the carbon footprint of industries and vehicles. Biofuels can be obtained from microalgae with the advantage of not competing for space with corn, sugar cane or other crops for food industry. Even though attractive, the biofuel production from microalgae presents some challenges, as for example the separation process required to obtain microalgae biomass. The culture is very diluted and the dewatering must be efficient, low cost and cause no damage to the cell. With the intent to address this issue, the herein paper presents a study of an alternative way to increase flocculation efficiency according to the temperature of the culture with the potential to improve the filtration efficiency in a continuous process. An increasing in the flocculation temperature from 20°C to 60°C increased the flocculation efficiency from 97.79% to 98.64%, using ferric chloride as a flocculant agent.

Keywords: microalgae, flocculation, microalgae harvesting, biofuel, biodiesel.

NOMENCLATURE

A	mean absorbance of clarified product
A_0	mean absorbance of microalgae culture

Greek symbols

η_{Abs}	flocculation efficiency

INTRODUCTION

The growing need to promote the development of alternative renewable sources of energy that have low environmental impact has attracted many researchers around the world to investigate biofuels derived from microalgae. Microalgae have great advantages over traditional crops, such as sugar cane, corn and soybeans. Microalgae have the highest production per area, up to 130 times higher than soybeans (Chisti, 2007) and has the great advantage of having no competition with the food industry, as other traditional sources. Another advantage is that photobioreactors can have a vertical type construction, which can maximize the volume per area (Satyanarayana, 2011). It also can be used in

other applications besides biomass production, such as sewage treatment and flue gas fixing.

In order to meet environmental concerns, Brazil seeks to implement legislation that encourages the production of biodiesel, placing a certain mandatory percentage of biofuel in diesel. In Brazilian diesel, 12% is biodiesel, and this percentage will increase by 1% each year until reaching 15% in 2023 (CNPE, 2018). This points towards an increasing demand of biodiesel, which rises the need to look for alternative sources that complement the production of biodiesel necessary to meet the legislated requirement.

Unfortunately, the biofuel production technology has not yet reached economic viability. One reason is the process of obtaining microalgal biomass, which has emphasis on dewatering of microalgal cultures. Some authors like Uduman et al. (2010) treat this process as the major bottleneck in the production of microalgae biofuels due to the large amount of water in the cultures.

DEWATERING MICROALGAL CULTURES

The challenge of obtaining microalgae biomass is due to parameters such as: i) low concentration of cells in the crop, which ranges from 0.2 to 0.6 g/L on average (Uduman, 2010); ii) cell size, which varies according to the species but it is common to find values between 3-30 μm (Grima, 2003); iii) cell density, which is very close to the medium, and iv) the electrical repulsion between the negative charged surface of the cells (Pugazhendhi et al., 2018).

There are several studies (González-Fernández, 2013; Vandamme, 2013; Mo, 2015; Chatsungnoen, 2016; Singh, 2018) in this field comparing several unit operations as for example, flocculation, flotation, centrifugation and sedimentation, in addition to possible combinations among them. However, there is no operation with great prominence that can be considered ideal. Thus, the choice ends up depending a lot on the conditions of the cultivation and, mainly, on the microalgae species.

Centrifugation is known for high cost operation and the damage on the cell wall caused by centripetal forces. Sedimentation, on the other hand, is a very slow process. The cell is very small and the difference in density between the particle and the fluid is also low, making the particle to take too long until reaching the bottom of the container. Dispersed air flotation can be used as a low-cost process, but it is difficult to control and reach the optimal condition of air flow and bubble size.

In order to minimize the costs of producing microalgae biofuels, a low energy cost technique such as flocculation is herein investigated. For this study, several compounds already known by the water treatment industries will be used, such as chitosan, aluminum sulfate ($Al_2(SO_4)_3$) and ferric chloride ($FeCl_3$). After flocculation and sedimentation of the biomass, this mixture still needs to go through more separation steps, such as centrifugation and filtration, which will be much easier by increasing the size of the microalgae flocs. Finally, the biomass will undergo through a drying process, which demands high energy expenditure. To minimize the costs of this last step, it is essential that the previous steps are able to remove as much water as possible from the biomass.

A study from Lemos et al. (2018) with Acutodesmus obliquus cultures (also known as Tetradesmus obliquus) shows a 96.8% efficiency of biomass recovery in ferric chloride flocculation process against 91.7% with centrifugation. This same work also reveals that a use of ferric chloride as a flocculant agent does not reduce lipid content, as with sodium hydroxide. This may indicate a cell rupture which explain the loss of lipids.

According to Wyatt et al. (2011), the mechanism of flocculation with ferric chloride can be explained with the presence of carboxyl groups (COO^-) on the surface of microalgae cells, which have negative charge. The ferric chloride solution decreases de pH of the microalgae culture, increasing the concentration of H^+ ions that combine with the carboxyl group, neutralizing the charge in the surface, promoting cells coagulation and, therefore, flocculation.

As an alternative to increase flocculation efficiency, we verify the possible effects of temperature on the flocculation unit operation using a solution of ferric chloride as flocculant in a culture of Tetradesmus obliquus. To identify these effects, parameters such as, absorbance, pH and biomass concentration were measured to compare the flocculation efficiency changing the temperature of flocculation, from 20°C to 60°C.

MATERIALS AND METHODS

All the experiments were conducted using the structure of the Research and Development Nucleus for Sustainable Energy (NPDEAS), this research center has a microbiological laboratory specialized in microalgae growth.

Microorganism and Cultivation Conditions

For this experiment the Tetradesmus obliquus were cultivated for 14 days in a controlled room where the temperature was kept constant at 22ºC with artificial light 24 hours a day. The microalgae cultivation was prepared in a 2 L erlenmeyer with 200 mL of microalgae culture from the NPDEAS and 1.4 L of synthetic medium culture Chu (Chu, 1942) Tab. 1 shows the list of components of the medium with their respective concentration. The culture received continuous injection of atmospheric air of 1.5 L/min providing gas exchange and agitation, preventing the cells from accumulate in the walls of the erlenmeyer.

Table 1. Chu culture medium composition.

Components	Concentration ($g.L^{-1}$)
Sodium Nitrate	0.250
Calcium chloride dihydrate	0.025
Magnesium sulfate heptahydrate	0.075
Monobasic potassium phosphate	0.075
Dibasic potassium phosphate	0.175
Sodium chloride	0.025
Ethylenediamine tetraacetic acid	0.050
Potassium hydroxide	0.031
Ferrous sulfate heptahydrate	0.005
Boric Acid	0.01142
Zinc sulphate heptahydrate	$8.82x10^{-5}$
Manganese chloride tetrahydrate	$1.44x10^{-5}$
Sodium molybdate dihydrate	$7.10x10^{-6}$
Copper sulfate pentahydrate	$1.57x10^{-5}$
Cobalt nitrate hexahydrate	$4.9x10^{-6}$

Cells Count

An optical microscope and a Neubauer's chamber were used to conduct the counting of cells, showing the number of cells per volume of culture. A sample of 1 mL was placed via capillarity in the Neubauer's chamber, the counting was made in the four corners squares and the center square of the chamber, the result multiplied by 5. This process was repeated in the two mirrors of the chamber, also a third count was made counting 5 randomic squares and multiplying the result by 5. The counting chamber with the sample can be seen in Fig. 1.

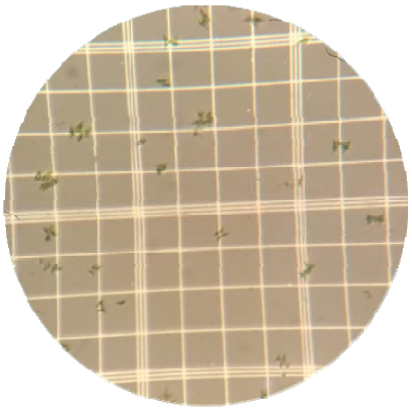

Figure 1. Neubauer's chamber with *Tetradesmus obliquus* sample.

Flocculation

In a bequer, 1 L of the microalgae culture was collected for all the experiments. 15 bequers of 50 mL were filled up with 40 mL samples from the 1 L bequer. The 15 bequers were divided in 5 groups of 3 samples each. The groups were named A, B, C, D and E and the three bequers of each group were numbered from 1 to 3.

Sample A1 was placed in a water bath setted at 20ºC, the sample remained there until it reached thermal equilibrium with the bath. After that, 12 mL of ferric chloride (1 g/L) were added to the bequer, resulting in a solution with 0.231 g/L of ferric chloride, the mixture was agitated with a glass rod for homogenization and then settled to rest for 5 minutes for decantation of the biomass.

After the decantation process, a sample of 10 mL was collected from the upper half of the bequer (the clarified solution) and stored in a flask with cap also labelled with the same name as the bequer sample, this sample was used for the absorbance tests. Another 10 mL sample was collected from the clarified solution in a 10 mL graduated cylinder, this sample was used for the biomass measuring.

This process was repeated two more times with samples A2 and A3. After that, the temperature of the water bath was adjusted to 30ºC and the flocculation and decantation process was repeated for triplicate B. The temperature was adjusted to 40ºC for triplicate C, 50ºC for triplicate D and 60ºC for triplicate E.
A schematic diagram of this flocculation process is shown in Fig. 2.

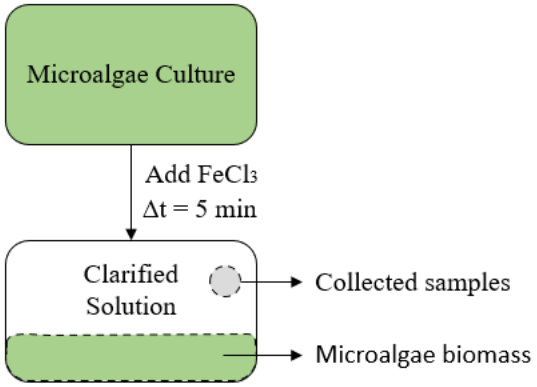

Figure 2. Schematic diagram of the flocculation process.

Dry Biomass

To obtain the dry biomass of the clarified solution and also the dry biomass of the microalgae culture, fiberglass membranes where dried for 24 hours in an air dryer oven set at 60ºC. The membranes where placed in a vacuum chamber to avoid humidity and after that, the membrane masses were measured in a high precision scale.

The 10 mL sample collected from the clarified solution in the graduated cylinder was used to obtain the dry biomass. The sample pass through the membrane in a Büchner funnel with a vacuum pump,

the membrane is placed back in the air dryer oven for 24 hours at 60ºC temperature for completely removal of water.

The dried membrane containing only dry biomass was placed in the vacuum chamber and the mass was measured in a high precision scale. Then the mass of the dried membrane is subtracted from the mass of the dried membrane with biomass, the result is the total dry biomass present in 10 mL sample.

A dry biomass analysis was also made for the microalgae culture before the flocculation process. Figure 3 show three samples of dry membrane with microalgae biomass after measured in the high precision scale.

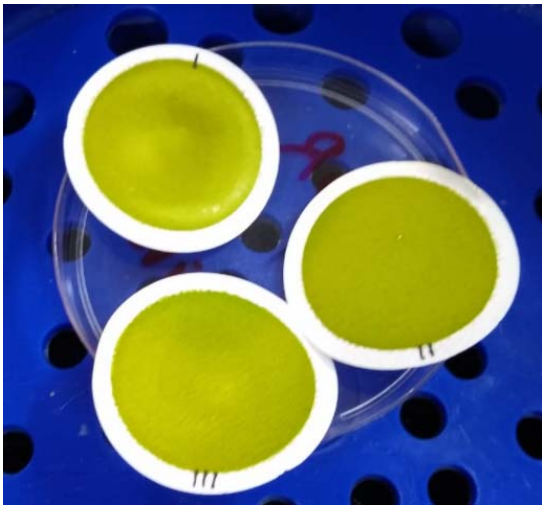

Figure 3. Microalgae dry biomass.

Absorbance

The Lambert-Beer's Law stated that more concentrated solutions absorbs more light, therefore to check the effectiveness of this flocculation an absorbance analysis was made. The lower absorbance values were directly proportional to lower concentrations of suspended solids (microalgae), then, in this case, the closer to 0, the better the flocculation efficiency.

A spectrophotometer (Perkin Elmer, model Lambda 25) was used to measure the absorbance. The spectrophotometer was configured with wavelength of 540 nm and quartz cuvettes. Deionized water was used for blank reading. The flask samples were used for absorbance analysis, for each sample 3 readings of absorbance were made.

An absorbance analysis was also made for the microalgae culture before the flocculation process. For that analysis, a dilution factor of 0.2 was used, so that the absorbance read in the equipment must be multiplied for the inverse of the dilution factor.

pH

The pH was measured by digital pHmeter (Gehaka, model PG1800) with an Ag/AgCl2 electrode at room temperature. The pH analysis was performed last, using the solution that was left in the 50 mL bequer to guarantee that the electrode would not agitate the decantated sample compromising the other tests. The pH measurement was also performed for the microalgae culture before the flocculation process.

Culture Data

A sample of this culture was analyzed to obtain the cell count, the pH, the absorbance and the total biomass. The data from this culture are shown in the Tab. 2.

Table 2. Data from *Tetradesmus obliquus* culture used in the experiments.

Cells count (10^4 cells/ml)	1075
	890
	835
Mean Cells count	933
Absorbance(1)	2.4105
	2.4300
	2.3795
Mean Absorbance	2.4067
Dry Biomass Concentration (g/L)	0.00857
	0.00891
	0.00941
Mean Dry Biomass Concentration	0.00896
pH	9.26

(1)multiplied by the inverse of the dilution factor.

RESULTS

A sample from the culture shown in the Tab. 2 was heated up to 20ºC (triplicate A), 30ºC (triplicate B), 40°C (triplicate C), 50°C (triplicate D) and 60ºC (triplicate E) and the results of the triplicates (I, II and III) are shown in the Tables 3, 4, 5, 6 and 7 containing the absorbance of the clarified (upper half) product, mean absorbance, the final pH of the solution and dry biomass concentration of the clarified product data.

Table 3. Triplicate A at 20°C: Experimental results of absorbance, final pH, and dry biomass concentration of the clarified product.

Triplicate A at 20°C	I	II	III
Absorbance	0.0611	0.0428	0.0512
	0.0616	0.0438	0.0541
	0.0654	0.0463	0.0522
Mean Absorbance	0.0627	0.0443	0.0525
Final pH	5.54	5.42	5.37
Dry Biomass Concentration (g/L)	0.042	0.018	0.022

Table 4. Triplicate B at 30°C: Experimental results of absorbance, final pH, and dry biomass concentration of the clarified product.

Triplicate B at 30°C	I	II	III
Absorbance	0.0423	0.0422	0.0472
	0.0428	0.0423	0.0432
	0.0461	0.0408	0.0507
Mean Absorbance	0.0437	0.0418	0.0470
Final pH	5.20	5.17	5.12
Dry Biomass Concentration (g/L)	0.042	0.015	0.019

Table 5. Triplicate C at 40°C: Experimental results of absorbance, final pH, and dry biomass concentration of the clarified product.

Triplicate C at 40°C	I	II	III
Absorbance	0.0437	0.0409	0.0405
	0.0430	0.0401	0.0394
	0.0431	0.0395	0.0410
Mean Absorbance	0.0433	0.0402	0.0403
Final pH	5.14	5.16	5.16
Dry Biomass Concentration (g/L)	0.023	0.046	0.025

Table 6. Triplicate D at 50°C: Experimental results of absorbance, final pH, and dry biomass concentration of the clarified product.

Triplicate D at 50°C	I	II	III
Absorbance	0.0341	0.0314	0.0498
	0.0356	0.0352	0.0503
	0.0323	0.0350	0.0438
Mean Absorbance	0.0340	0.0339	0.0480
Final pH	5.15	5.12	5.13
Dry Biomass Concentration (g/L)	0.027	0.026	0.015

Table 7. Triplicate E at 60°C: Experimental results of absorbance, final pH, and dry biomass concentration of the clarified product.

Triplicate E at 60°C	I	II	III
Absorbance	0.0352	0.0300	0.0352
	0.0345	0.0237	0.0347
	0.0341	0.0304	0.0358
Mean Absorbance	0.0346	0.0280	0.0352
Final pH	5.04	5.02	5.09
Dry Biomass Concentration (g/L)	0.035	0.021	0.019

To identify possible outliers on the data, we use a Dixon's Q test at 90% of confidence level. With this test were disregarded the points of absorbance in the sample I at triplicate C and sample III at triplicate D. Those points are not considered in the graphics ahead since the test defined these points as an abnormal result.

Absorbance

According to Lambert-Beer's law, the absorbance is directly proportional to the concentration of a solution, so we can use the absorbance to verify the behavior of the microalgae cells concentration in the clarified product versus the temperature, which is shown in Fig. 4 bellow.

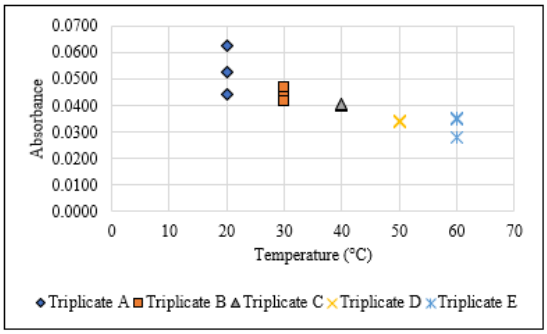

Figure 4. Mean absorbance of clarified product versus temperature.

In Fig. 4 is possible to observe a trend in which the higher the temperature, the lower the absorbance. So, we can say that the higher the temperature of the microalgae sample, the lower the microalgae cells concentration. That can be interpreted as a better flocculation, or higher flocculation efficiency.

To calculate the efficiency of the flocculation using absorbance, we can use the Eq. (1) below, where $\eta_{Abs}(\%)$ is the flocculation efficiency, A is the clarified product mean absorbance and A_0 is the absorbance of the microalgae culture, before the flocculation. The results of the flocculation efficiency are shown in Tab. 8.

$$\eta_{Abs}(\%) = \left(1 - \frac{A}{A_0}\right) \times 100 \quad (1)$$

Table 8. Flocculation efficiency in different temperatures using mean absorbance.

Temperature (°C)	Flocculation Efficiency (%)
20	97.79
30	98.16
40	98.29
50	98.40
60	98.64

pH

In the Fig. 5 we can observe another trend, the samples with higher temperature, which were the ones with the highest flocculation efficiency, are those with the lower pH. This can be explained considering the neutralization of the electric charge from the surface of the microalgae cells that occurs in the presence of H^+ ions, which is higher in lower

values of pH. Then a higher surface charge neutralization implies in a better flocculation efficiency. Furthermore, there is also the effect of temperature in pH since the increase of temperature decreases the pH by disturbing the water chemical equilibrium (Le Châtelier's principle).

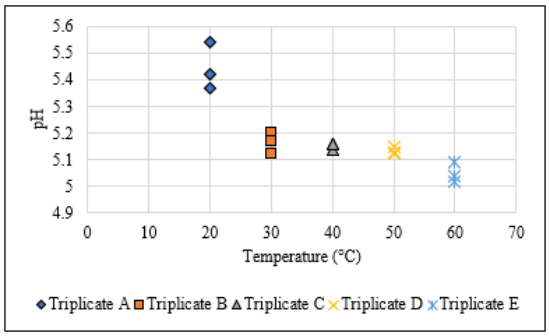

Figure 5. The final pH of the solution versus temperature.

Dry Biomass Concentration

In addition to this study, we calculate the mean dry biomass concentration to determine the amount of biomass present in the clarified product that is not flocculated. The results are shown in Fig. 6 below.

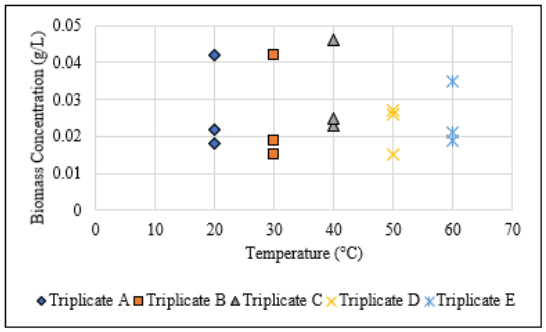

Figure 6. Mean dry biomass concentration from the clarified product versus temperature.

The results showed a slight downward trend, what demonstrates a better flocculation, since the lower the concentration of biomass in the clarified product, more biomass has flocculated and decanted. That suggests a better flocculation efficiency. On the other hand, this trend is not as clearly as in previous ones. One way of explaining this may be the very low concentration of biomass that is close to the error of the method, since the absorbance analysis showed that the concentration of cells in the clarified product is low (less than 3%). In addition, the clarified product still has a few small flakes that have not decanted, which shows that this solution has a concentration of solids that is not homogeneous.

CONCLUSIONS

In this work we could observe that increasing the temperature of the flocculation process, it was possible to increase the flocculation efficiency, from 97.79% at 20°C, to 98.64% at 60°C. Although this difference may be small, it opens the possibility of studies in this area involving other species of microalgae and other types of flocculants. It must also be kept in mind that this is only one step in the process, and other unit operations such as filtration, may be favored with the increase of temperature.

As stated earlier, dewatering microalgal cultures is the major bottleneck in the process, that can determine the economic viability of the microalgae-based biofuels, so a small efficiency increase can reduce the operation cost and make it viable.

Although the temperature of the microalgae cultivation reactors cannot be changed due to the optimal conditions for the growth of microalgae, it is possible to add a heat exchanger to increase the inlet temperature of the harvesting process and obtain a better separation of microalgae biomass in the flocculation. The heat source can be an energy reuse, since a small increase in temperature already results in better efficiency in flocculation. However, the same effect must be evaluated in subsequent operations, observing if there are variations in the performance of that operation.

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