DENSITY CONTROL OF MICROALGAL CULTURES USING INFRARED SENSORS

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Abstract. *Photobioreactors are closed microalgae cultivation systems that show several advantages in comparison with traditional cultivation systems like pools and raceway pounds. Tubular photobioreactors basically consist of clear tubes where the medium circulates in the presence of natural or artificial light. Air or CO_2 enriched air mixture can be introduced in the reactor increasing the using of this carbon source in the photosynthesis process and, consequently, getting higher biomass production. The Center for Self-Sustainable Energy Research & Development (UFPR) proposes the production of biodiesel from microalgae oil. The cells will be cultivated in horizontal tubular photobioreactors and, after harvesting and drying the biomass, the separated lipid will be used to manufacture biofuel. The production will be in a semi-continuous culture system through simultaneous addition of new medium and harvesting of culture. The system must be adjusted to process the dilution when the cells in the culture achieve a previously specified concentration. An apparatus for optical density determination was designed, and consists of an array of near infrared light emitting light dependent resistor positioned on the clear tube in the opposite side of a phototransistor detector. The total transmitted light will be converted to Resistance (Ohm) and related to biomass ($g.L^{-1}$) or cell concentration (cells. mL^{-1}). The calibration is possible through simultaneous experiments such as the counting of microalgae cells in Neubauer chamber under microscope and gravimetrical determination of biomass.*

Keywords*: microalgae, photobioreactor, biodiesel, light dependent resistor, infrared sensor.*

1. INTRODUCTION

The applications of microalgae have been the subject of several studies in recent decades. Its commercial uses are diverse, such as food, animal feed, cosmetics, aquaculture and production of chemical compounds of high added value (pigments, fatty acids, nutritional supplements, etc.) (Spolaore *et al.* 2006). Considering the many species of algae exist, these organisms may be source of a wide variety of products and applications. World production of biomass of microalgae is around 5000 t / year of dry matter. Success in the production of microalgae depends on the species chosen and the culture conditions (Pulz and Gross, 2004).

Phaeodactylum tricornutum (UTEX 640) is a species of microalgae belongs to the class *Bacillariophyceae* (diatoms) and are rich in eicosapentaenoic acid (EPA) (Yongmanitchai and Ward, 1991).

Recently the substitution of fossil fuels as primary energy matrix is world widely recognized because of various environmental impacts caused by this and be a non-renewable source. An alternative to diesel oil is the biodiesel. The biodiesel plant is a fuel oil in potential, because it is neutral in carbon emissions and renewable, however, impeding the progress of this source is the large area required for the production of oil that does not meet the current need for oil. A solution to this is the biodiesel from microalgae. Like the plants, the microalgae use light as an energy source for the production of oil and have a higher percentage by weight of oil that the oil plants. The technology for production of biodiesel is known for more than 50 years, but that biodiesel was produced mainly for soybean oil (Chisti, 2007).

The production of biodiesel from microalgae became viable because of its great capacity of energy transformation. The microalgae can contain large amounts of lipids in their biomass, exceeding the best oil plant. This lipid is rich in fatty acids C16 and C18, being produced in your photosynthetic activity and quantity can vary between 20% and 80% of the composition of the biomass of algae (Song *et al.* 2008).

The microalgae can be cultivated in various forms, as in open tanks or photobioreactors. Photobioreactors consist of a chain of transparent tubes arranged in parallel where the cultivation media circulate with the help of a pump. His arranged offers a closed culture with less risk of contamination maintains a minimum contact with the environment. To keep the photobioreactor operating continuously is needed injection of CO_2 and the removal of O_2 from photosynthesis of algae (Grima *et al.* 2000).

To further exploit the photobioreactor is needed to establish the moment in which the means of cultivation reached the end of exponential time, in other words, the point where there is stabilization in the multiplication of cells. At this

point is the period that the growth rate of the medium is equal to the rate of dying cells. It is very important to know this point because this is the time where the growth should be stopped since it reached the maximum production of algae.

There are several methods to determine the number of cells in a population of microalgae and they can be classified into three categories: optical, mechanical and electrical. One of the non-evasive methods most used is the measure of the turbidity of the medium through of devices known as turbidimeters (Yamana, 1993).

The aim of this paper is mounting an optical sensor that is able to determine in real time the number of cells of microalgae that through a tube with a minimum of external interference in the system.

2. MATERIAL AND METHODS

2.1. Culture Media

The marine microalgae *Phaeodactylum tricornutum* was obtained from Integrated Group from Aquaculture and Environmental Studies (GIA) of the UFPR.

The starter cultures was prepared from strain to five flasks containing 250 mL of enriched sea water and were kept under constant aeration, temperature of 22 º C and artificial lighting of fluorescent lamps. After five days, this culture were inoculed into five Erlenmeyer flasks with a volume of 1 L containing enriched sea water and was kept on the same conditions for 5 days. After that, it was transferred to a tank of 500 L maintained inside a greenhouse under temperature and lighting environment. After 10 days of growth was removed one sample used for sensor calibration.

All cultures were grown in sterilized seawater and enriched with Guillard F/2 medium. This is a common and widely used general enriched seawater medium designed for growing coastal marine algae, especially diatoms. Into one liter of filtered natural seawater with salinity of 0.5%, was added the components showed in Tab 1.

Table1. Composition of Guillard F/2 medium.

Component	Stock Solution (g/L dH_2O)	Quantity Used
$NaNO_3$	75	1 mL
$NaH_2PO_4 \cdot H_2O$	5	1 mL
$Na_2SiO_3 \cdot 9H_2O$	30	1 mL
Trace metals solution	See Tab 2	1 mL
Vitamins solution	See Tab 3	0,5 mL

Table 2. Trace metals solution .Into 950 mL of distillated water (dH_2O), dissolve the EDTA and other components. Bring the final volume to 1 liter with distillated water (dH_2O)

Component	Stock Solution (g/L dH_2O)	Quantity Used
$FeCl_3 \cdot 6H_2O$	-	3,15 g
$Na_2EDTA \cdot 2H_2O$	-	4,36 g
$MnCl_2 \cdot 4H_2O$	180	1 mL
$ZnSO \cdot 7H_2O$	22	1 mL
$CoCl_2 \cdot 6H_2O$	10	1 mL
$CuSO_4 \cdot 5H_2O$	9,8	1 mL
$Na_2MoO_4 \cdot 2\ H_2O$	6,3	1 mL

Table 3. Vitamins solution. Into 950 mL of dH2O, dissolve the thiamine · HCl, and add 1 mL of the primary stocks. Bring final volume to 1 liter with dH2O. Filter, sterilize and store frozen.

Component	Stock Solution (g/L dH_2O)	Quantity Used
Thiamine · HCl (vitamin B_1)	-	200 mg
Biotin (vitamin H)	1,0	1 mL
Cyanocobalamin (vitamin B_{12})	1,0	1 mL

2.2. Construction of Infrared Sensors

The sensor was mounted and constructed externally on one transparent PVC pipe with external diameter of 2.54 cm. The electronic circuit for optical density determination consists of an array of six infrared light emitting diodes (LEDs) with a wavelength of 800 nm. The infrared light emitted by LEDs is captured on the opposite side of the tube by a LDR (Light Dependent Resistor). This system was placed inside a closed black box to minimize the external interference and

protect the circuit from possible accidents. The number of LEDs and your distribution in the circuit was selected in order to give high instrument sensibility. The LDR is an electronic component whose electrical resistance decreases when luminous energy shines onto the LDR, in other words, the resistance of the LDR decreases as the intensity of the light falling on it increases. Therefore, the LDR resistance depends on the amount of light that focuses on its base after crossing the culture media. Knowing that the turbidity of the culture medium increases with the number of cells and that a change in turbidity causes a variation in the amount of light that through the tube, will be found a correlation between the number of cells of the microalgae cultivation and the resistance measured in LDR.

2.3. Calibration of Optical Density Sensors

The calibration of the sensor was performed with six dilutions of a sample from a culture after 10 days. These dilutions were prepared with sea water and their values are shown in Tab 4. The cell concentration of each dilution was determined using a Neubauer chamber and a microscope. This counting is done taking a small sample of the culture medium of you want to determine the number of cells and place it in a Neubauer chamber where it is possible with the aid of the microscope to count the number of cells present in a small volume pre-determined by the camera.

Table 4. Six dilutions realized in this experiment.

Number	Volume of Medium (mL)	Volume of Water (mL)	Total (mL)
01	100	0	100
02	80	20	100
03	60	40	100
04	40	60	100
05	20	80	100
06	0	100	100

After the counting, was introduced the solution of microalgae in the sensor and measured the resistance of the LDR with the help of a digital multimeter. An each new solution introduced in the sensor to measure the resistance has been taken care that there were not residue of previous solution inside the sensor and its ends were covered for that there was not interference from any source outside the sensor.

3. RESULTS AND DISCUSSION

The optical sensors were calibrated to cell concentration in function of resistance of LDR. Two experiments were prepared with the same sample of algae, producing similar results. The results of analysis of the number of cells per mL present in each dilution are shown in Table 5. Looking at the figures it can be seen clearly that the resistance of LDR increases proportionately with the quantity of cells, thus we can affirm that the turbidity of the medium depends on the number of cells and the turbidity difficulty the passage of light through the tube, making the LDR receives less light and change their resistance.

Table 5. Results of sensor calibration.

1º Experiment			
Volume of Medium (mL)	Volume of Water (mL)	Cell Concentration (cells. mL^{-1})	Resistance (MΩ)
100	0	2120000	68,8
80	20	1920000	62,8
60	40	1500000	60,7
40	60	1120000	55
20	80	520000	51,2
0	100	0	44,3
2º Experiment			
Volume of Medium (mL)	Volume of Water (mL)	Cell Concentration (cells. mL^{-1})	Resistance (MΩ)
100	0	2090000	68,1
80	20	1550000	64,8
60	40	1460000	64,3
40	60	1000000	57,4
20	80	530000	49,5
0	100	0	46,7
3º Experiment			
Volume of Medium (mL)	Volume of Water (mL)	Cell Concentration (cells. mL^{-1})	Resistance (MΩ)
100	0	1890000	68,9
80	20	1630000	65,5
60	40	1440000	56,4
40	60	880000	54,2
20	80	560000	50,4
0	100	0	43,2

With the data presented in Tab 5, we built a graph shown in Fig 1 with the variation of resistance in function of the number of cells.

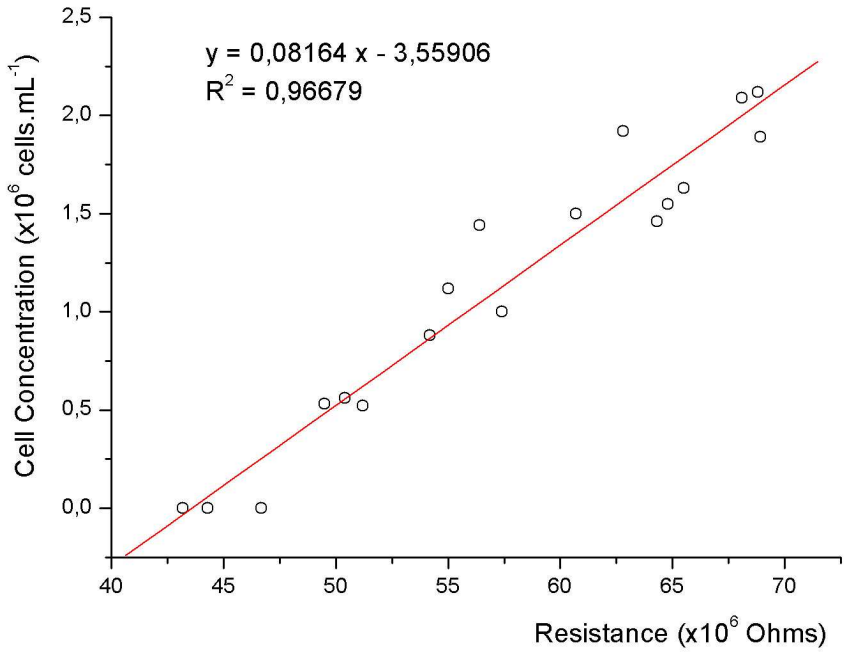

Fig 1. Cell concentration in function of Resistance.

The results obtained indicate a linear tendency between cell concentration and the resistance of LDR. Because it, was built a linear regression in order to obtain an expression to calculate the number of cells of cultivation media. It was observed that for lower values of number of cells was found results very close to the resistance, however, there was a diversion to high cell concentrations. Even with this dispersion for high values, we can conclude that the resistance varies linearly for any cellular concentration of the medium. It is important to emphasize each sensor must be individually calibrated.

4. CONCLUSIONS

The results showed that it is possible to calculate the cell concentration in a medium using an optical sensor that determines the quantity of light through the medium. Although the values found are not as close to the calibration curve, however the sensor can still be used to determine the cellular concentration of a cultivation medium because the objective of photobioreactors is the maximum algae biomass production per day and that sensor allows us a good approximation of the real value.

This study showed the possibility to construct a single sensor, with minimal external interference in the system, low cost and allowing a real-time monitoring the growth of culture medium, can replace the other methods for determining cell concentration.

5. REFERENCES

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5. RESPONSIBILITY NOTICE

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