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Microalgae *Scenedesmus sp* as a Clean Technology in reducing Greenhouse Gas Carbon Dioxide

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Currently, the use of clean technologies is being sought as a more natural and environmentally friendly strategy and alternative to reduce the concentration of carbon dioxide (CO_2) in the environment in order to decrease the presence of this greenhouse gas that is harmful to the weakening of the ozone layer. One of these methods is the use of microalgae, which are photosynthetic microorganisms, capable of fixing this gas. The use of microalgae has advantages such as a minimum impact on the environment, it does not require a productive soil or clean water, from the microalgae is also generated biofuels and especially has the capacity to absorb CO_2. The investigation had as purpose to determine the biocaptation of Carbon dioxide of the microalgae *Scenedesmus sp*. The method of test consisted of generating CO_2 in the environment inside water with the alga in mention for a space of 12 days being able to reduce CO_2, of an initial concentration of 0.002640 mg/L to 0.00556 mg/L, being corroborated that the microalgae *Scenedesmus sp*. is effective for biocaptation of Carbon dioxide. The cell density of the microalgae *Scenedesmus sp*. in the optimum treatment was of an initial number of cells of 1.200.000 cells/mL and ending with a total of 1.602.000 cells/mL with the temperature in the range of 22 °C to 27 °C, showing a stability and where there were no problems with the adaptation medium.
Keywords*: CO_2 reduction, biotechnology,* microalgae *Scenedesmus sp*.

1. Introduction

Global warming has been causing concern throughout the world, the consequences caused by greenhouse gases are increasing, one of the main gases is Carbon Dioxide that currently increases constantly, weakening the ozone layer (Oscanoa et al., 2015). In recent years, various anthropogenic factors are the industrial development such as the automotive system, which are managed by economic growth have been evidenced (Ríos et al. 2016). In Peru, a projection was made in 2012 on greenhouse gas emissions for the period 2011 to 2018, indicating that in the last year 218.7 million tons of these polluting gases would be emitted into the atmosphere, including carbon dioxide, methane, and oxides nitrous (SINIA, 2012). At present, the levels of carbon dioxide emissions are increased, as a consequence, the increase in temperature is generated that causes a modification of the climate causing irreversible damage, this brings with it economic, environmental, social and political instability (Guzmán et al., 2016). It was estimated that there were 36.8 billion metric tons of carbon dioxide in the air in 2019. According to the UN's 2020 Emissions Gap Report, it indicates that despite the reduction in carbon dioxide emissions from the COVID 19 epidemic, it did not slow down the emission of greenhouse gases much and it will not be possible to meet the objectives of the Paris Agreement to limit global warming to below 2 °C. (UN, 2020).
This worrisome situation at present is reason to raise research, develop clean technologies and techniques that face environmental problems. At present, one of the studies of technologies and environmental innovation has focused on the use of microalgae, due to its great photosynthetic capacity (Diaz and Restrepo, 2011).

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The use of microalgae, due to the efficient work carried out in the remediation mechanisms of liquid and gaseous pollutants, contributes to the sustainable energy generation system. (Hernández and Labbé, 2014). The study of microalgae has motivated the interest in reducing CO_2 emissions, through photosynthetic processes that manage to capture carbon dioxide 10 to 50 times greater than the photosynthetic uptake of land plants (Barajas et al., 2012).
In carbon dioxide capture research, the concentration potential that a microalgae can support, adaptation and biomass production is evaluated (Sandoval and Rubio, 2017). The evaluations regarding the uptake of $_{CO2}$ and photosynthetic efficiency, the microalgae *Scenedesmus sp*. tolerating 80%, *Chlorella sp* presenting 40% and *Nannochloris sp* showing 15%, CO_2 uptake (Gaikwad et al. 2016). The cultivation of microalgae has physical requirements for their life, which are temperature, pH and photoperiod (Barajas and Sierra, 2017). The benefit provided by microalgae is already known as sources of biodiesel and bioethanol and with a good prospect for generating energy (Peralta et al., 2018).
The purpose of the research was to demonstrate that the application of the microalgae *Scenedesmus sp* for the fixation of carbon dioxide as an alternative of sustainable and environmentally friendly technology. The main physicochemical parameters such as pH, temperature and cell density were also evaluated for the optimal development of the microalgae.

2. Materials and methods

2.1 Materials

For the inoculation of the microalgae *Scenedesmus sp*, was carried out in Erlenmeyer flasks of 500 mL, an air pump was installed to oxygenate it, silicone hoses, Bayfolan liquid foliar nutrient of Bayer company and a lighting system. 2 L volume containers were used for CO_2 sources.
The microalgae *Scenedesmus sp* was acquired from the Instituto del Mar del Perú (IMARPE), the Peruvian Maritime Resources Research Institution.

2.2 Carbon dioxide capture procedure

Inoculation of the microalgae *Scenedesmus sp*
It was performed using 8 mL of *Scenedesmus sp* microalgae in an Erlenmeyer flask, 492 mL of mineral water was added to achieve a volume of 500 mL of culture, 0.07 mL/L of Bayfolan foliar nutrient was applied to seek and achieve the growth of the microalgae. The cultivation system was adapted to a bubble aeration system to have a better circulation of nutrients and avoid strata. The temperature and pH were controlled daily, the lighting conditions were 12 hours of light projection and 12 hours of dark phase, the cell density was performed every six days to show the progressive growth of the microalgae (Figure 1)

Figure 1: Scenedesmus sp microalgae culture system

The pH determines if the culture medium is soluble in CO_2, estimates the oxidation of minerals and part of the metabolism of the microalgae. The optimum pH range of *Scenedesmus sp* varies between 5.6 to 7, this depends on the acidic or basic medium so as not to generate crop collapse (Huancollo, 2018).
The use of the foliar nutrient bayfolan requires an increase in pH in order to improve the capacity of cellular nutrition, in such a way that if it is necessary to increase the acidity of the culture medium, sodium hydroxide at 10% is added for each volume of 150 mL and to decrease the acidity of the mineral water should use acetic acid to generate a balance in the crop.

Carbon Dioxide Generation

For the generation of the carbon dioxide source, 10 g of fresh yeast, 500 g of sugar and 1 L of water were used. The process was developed in an anaerobic medium to generate a uniform fermentation of 4 samples as shown in Figure 2.

Figure 2: Carbon dioxide generation

Capture Carbon Dioxide

2 mL of solution with *Scenedesmus sp*. Microalgae was applied. to each fermentation sample to assess carbon dioxide capture in the period of one week. In this period of time, dissolved oxygen (DO) was evaluated in the 4 samples (with 3 repetitions), as shown in Figure 3.

Figure 3: Dissolved Oxygen Control in the Scenedesmus sp microalgae fermentation system

3. Results and discussion

3.1 Cell count

According to Table 1, the result of the evaluation of the cell count of the 4 treatments is presented in an exponential phase process, it is evidenced that the conditions of the culture medium and foliar nutrition an efficient development adaptation of the microalgae *Scenedesmus sp*. Optimal growth conditions are essential to achieve accelerated reproduction, taking into account pH, temperature, light intensity, aeration and foliar nutrition (Pedraza and Prada, 2018). The application of the foliar nutrient (Bayfolan of Bayer) is a concentrated formula of nutrients and vitamins that activate metabolic processes of microalgae, contain macro and micronutrients.

Table 1: Cell count of the microalgae Scenedesmus sp.

Sample	Initial cell count (cel/mL)	Final cell count (cel/mL)
Treatment 1	1.200.000	1.602.000
Treatment 2	844.000	1.179.000
Treatment 3	698.000	983.000
Treatment 4	494.000	743.000

The cell count of each treatment was calculated over a period of 6 days until reaching the final cell count shown in Table 1. After inoculating the solution with *Scenedesmus sp* microalgae in the fermentation solution, the control was done weekly. To determine cell density, the use of the Neubauer Chamber is required, which facilitates evaluating the conditions of microalgae and their exponential growth (Ynga and Niño, 2019).

The formula for the Neubauer chamber is shown in Eq. (1), where

$$Cell\ concentration = \frac{Total\ cells\ counted}{Number\ of\ squares}\ x\ 100 \qquad \text{(Eq.1)}$$

3.2 Temperature

The evaluation of the temperature to each one of the treatments, determined a range between 22 °C to 27 °C, being an optimal temperature for the development of the inoculation process. If the temperature exceeds 35 °C, cell growth cannot be developed (Santos, 2014). The optimal development of algal production and growth consists that the temperature must maintain a balance according to the species, when exceeding it produces a series of difficulties, presenting an increase in cell death in the culture.

Table 2: Temperature measurement

Sample	Initial temperature (°C)	Final temperature (°C)
Treatment 1	26.7	26.0
Treatment 2	25.8	25.2
Treatment 3	25.6	25.1
Treatment 4	24.4	23.7

3.3 Hydrogen potential (pH)

The pH during the exponential phase of the microalgae *Scenedesmus sp* had an optimal range of 3.67 to 4.86 of the microalgae to develop the photosynthetic process. The pH condition in microalgae cultures is defined according to the species, where it can be maintained in equilibrium through aeration and CO_2, fulfilling the function of oxidation of nutrients. The pH balance ensures stability and reproduction of the culture to avoid high concentrations of CO_2 that turn into an acidic medium and this generates cell death (Castillo, 2020).

3.4 Carbon Dioxide Concentration

The initial and final concentrations of carbon dioxide in the 4 treatments are presented in Table 3. The efficiency of the microalgae *Scenedesmus sp* in CO_2 uptake was demonstrated, and it was found that treatment 1 was the most representative in CO_2 reduction with 75% (0.001981 mg/L). The evaluation of the 4 treatments was carried out over a period of 14 days: on day 1 the microalgae in question was applied to the fermentation medium and the remaining 13 days the CO_2 fixation photosynthetic activity of each treatment was evaluated. This is detailed in Table 3, this information is relevant, evidencing the fixation capacity of the microalgae and its differences in the period of time. There are studies that based on this type of information, researchers have projected to generate energy from CO_2 capturing algae, (Nurariffudin M., et al, 2017).
Algae are photoautotrophic eukaryotic living beings that perform photosynthesis, a process that transforms the energy of sunlight into chemical energy, consisting in the elaboration of sugars from CO_2, minerals and water. This is generated from the metabolization of carbon dioxide and water into oxygen and carbohydrates. Photosynthetic pigments (chlorophyll) absorb adequate light for photosynthesis.

Algae need a protein called RUBISCO, which transforms carbon dioxide into oxygen and carbohydrates, (Pedraza and Prada,2018) see Figure 4.

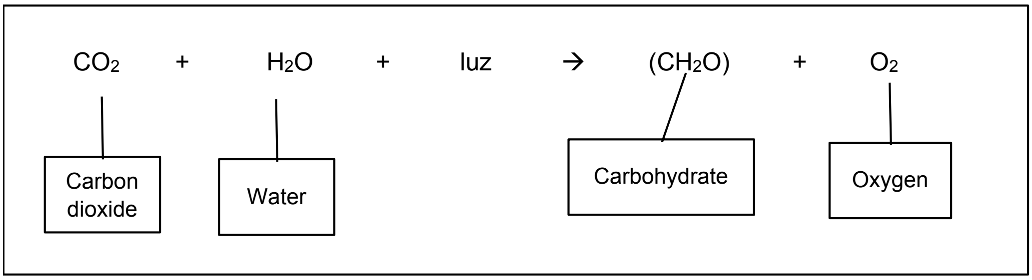

Figure 4: Photosynthesis process

The scientific theory indicates that in the air there are percentages of Oxygen (O) 20.94% and Carbon Dioxide (CO_2) 0.035%, the oxygen contributed by the microalgae was calculated by Eq. (2), the dissolved oxygen of the fermentation medium is evaluated at 100%, 20. 94% is the oxygen present in the air. 94% is the oxygen present in the air, performing a mathematical operation shows the oxygen contributed by Scenedesmus sp. The microalgae capture CO_2 through the photosynthesis process where the metabolization of CO_2 and water into oxygen and carbohydrates is generated. Scenedesmus sp, is applied in a fermentation medium with CO_2 production, the initial evaluation is performed and after the capture process the final evaluation of CO_2, these results were calculated using Equation (3), the oxygen provided by the microalgae is taken as 20.94%, the percentage of CO_2 is 0.035% in the air, a mathematical operation is performed and thus the concentration of carbon dioxide is obtained, which is presented in Table 3

Calculation of dissolved oxygen, taking into account that:

O_2 ---- 20.94%
CO_2---0.035%

$$\begin{array}{l} \text{Disolved oxygen} ----- 100\% \\ \quad X \quad ------20.94\% \end{array} \qquad \text{(Eq. 2)}$$

Where X is Oxygen provided by Scenedesmus sp

$$\begin{array}{l} \text{Percentage of } 21\% \text{ oxygen} \quad ---- \quad 20.94\% \\ \quad Y \quad -------- 0.035\% \end{array} \qquad \text{(Eq. 3)}$$

Where Y is concentration of CO_2

Table 3: Concentration de CO_2

Sample	Initial concentration (mg/mL)	Final concentration (mg/mL)	Concentration variation of CO_2	Reduction percentage of CO_2
Treatment 1	0.002640	0.000659	0.001981	75%
Treatment 2	0.001854	0.000820	0.001034	56%
Treatment 3	0.001592	0.000556	0.001036	65%
Treatment 4	0.001502	0.000618	0.000884	59%

4. Conclusions

The use of microalgae cultures turns out to be an environmental and eco-friendly way as a means of capturing carbon dioxide, one of the most abundant greenhouse gases in the air. It has the advantage of developing large-scale exponential growth and carbon dioxide binding capacity with efficient energy-sustainable processes. As a result of the research, it was established that the microalgae *Scenedesmus sp*, at optimal conditions of temperature between 22 °C to 27 °C and at pH in a range of 3.67 to 4.86, its growth was exponential. Likewise, it was found that for the treatment with the most efficient results, it had an ability to reduce carbon dioxide in 12 days from an initial value of 0.002640 mg/L to 0.000659 mg/L, by photosynthetic mechanism. This clean methodology has a lot of environmental perspective in solving the air pollution caused by carbon dioxide, a problem that is getting worse very quickly.

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