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Use of Microalgae Using Earthworm Leachate (Lumbricidae) for Application in Agriculture

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ABSTRACT

Microalgae have unique properties that allow them to thrive in unconventional spaces, making them suitable for areas that are not normally suitable for crop growth. This is due to their ability to multiply rapidly, grow easily and adapt to different environments at low cost. As a result, the present study aims to analyze the potential of microalgae as a source of agricultural nutrition, as well as the health benefits they can provide. The following research was conducted on an experimental scale using a closed system (photobioreactor) for the cultivation of microalgae, its physicochemical characterization of the cultures and dry biomass. It can be observed the percentages of 11 N; 1.4 P; 0.3 K and its micronutrients are essential for plant growth, since microalgae-based biofertilizers are considered as a sustainable, cost-effective and environmentally friendly alternative to chemical fertilizers. The use of microalgae biomass as a biofertilizer in agriculture can increase fertility, reduce soil erosion and nutrient loss, and improve soil quality over time. It also benefits plants, vegetables and greens, as it contains nitrogen, phosphorus and potassium, which are necessary for growth.

Keywords: agriculture, biofertilizer, biomass, photobioreactor, microalgae.

INTRODUCTION

Microalgae, part of the microscopic group, are photosynthetic and multi-faceted taxa, commonly known as Microalgae. They possess unique properties that allow them to prosper in unconventional spaces, and making them suitable in areas that are normally not suited for culture growth. This is due to their capability to multiply quickly grow easily and adapt to different environment with little effort (Odjadjare et al., 2017; Wang et al., 2014). Besides absorbing sun light and carbon dioxide, microalgae consume nutrients from the soil or from aquatic habitats, they are also an important source of oxygen in the atmosphere (Rizwan et al., 2018). Microalgae not only help to reduce greenhouse gas emissions by converting carbon dioxide into biomass, but they also possess great biotechnological potential for industrial purposes. Carbohydrates, proteins and lipids are the main components of algae biomass. The percentage of lipids can vary between. 4 and 61% Rhodes (2009), while for proteins it varies between 46 and 63% of dry mass (Garibay-Hernández et al., 2009). Carbohydrates often constitue10 a 17% (Farag et al., 2012).

The base structure of microalgae allows for rapid cell proliferation due to their photosynthetic nature. This enables them to transform carbon dioxide and light energy into valuable biomass that can be used for commercial purposes (Benavente-Valdés et al., 2014). Microalgae require specific circumstances for effective growth, such as light, temperature, pH, CO_2, and adequate nutrients. Light intensity is an important factor to determine the necessary photosynthetic activity for the production of biomass. Temperature can vary between species, but it normally ranges between 28 and 35 degrees Celsius (Park et al., 2011). The pH is influential in food availability, solubility. The growth and metabolism of algae. The optimal growth range from 7 to 9, while for freshwater species, 8 is preferable (Beltrán-Rocha et al., 2017). This depends on the environment of growth; therefore, it can vary. Nitrogen and phosphorus are the main required nutrients for growth. Nitrate and ammonium compounds are the most commonly used techniques for adding nitrogen supplements. As a limiting ingredient for biomass development and biological processes, phosphorus exists in the form of hydrogen phosphate (HPO_4) and phosphate (PO_4) (García, 2008).

These photobioreactors are adaptable systems that can be optimized depending on their geometry (tubular, cylindrical or flat) and design (plastic or glass). These kind of reactors allow for better control of culture conditions and growth parameters (pH, temperature, mix, CO_2 and O_2), reduce evaporation, CO_2 loss, increase microalgae density and volumetric performance (Gonzales, 2016; Ho et al., 2011).

The secretion of earthworms is a concentrated brown fluid that contains great quantities of microbial matter and nutrients (García, 2015). The solution is compatible with pressurized irrigation systems, which makes it adequate for organic agricultural production in greenhouses (Jaramillo et al., 2018). The leachate contains relatively high levels of nitrogen potassium and phosphorus. They are important for the growth of plants due to their efficiency for production (Bermeo, 2009).

Cadena (2014), proposes various properties and benefits that worm leachate offers: Increase soil microorganisms, foster growth from the root, extend the duration of soil moisture, makes plants produce more chlorophyll, decreases the conductivity of saline soil, increases pH of acidic soil, balances the growth of fungi in the soil, reduces the activity of plagues such as aphids, improves the efficiency of many fertilizers and commercial herbicides, it is absorbed through stomata and roots.

Microalgae weight can be composed of more than 88% water. Before it is commercialized, it must be dehydrated to retrieve the dry biomass. Sun drying is the oldest and most profitable method of marine algae conservation, having been used since antiquity. They are dried on a hot air oven (50 °C) for 6 hours until their weight becomes constant. The range of acceptable moisture is between 5% to 7% (Gamarra et al., 2018).

Nowadays, the pressing need to protect the environment and fight the negative effect of climate change in agriculture have resulted in a widespread and revitalized use of plant extracts and algae to prevent and treat plants, increase yields and treat illnesses. These extracts are biodegradable and have low or zero toxicity for plants and animals (Crouch and van Staden, 1993; Povero et al., 2016). The inadequate use of chemical products causes the loos of layers of fertile soil, biodiversity and the extinction of natural plagues (Battacharyya et al., 2015).

In agriculture, the microalgae can improve soil fertility, the development and protection of crops, while providing an alternative to fertilizers and chemical pesticides (Holajjer et al., 2013; Prasanna et al., 2015). Microalgae improve the soil's nitrogen cycle and can help plants grow with greater nutrient availability (Karthikeyan et al., 2007).

In this context, microalgae have the potential to be used in the development of organic biofertilizer, bio stimulants, biocontrol agents and conditioners for improving the soil, as well as crop protection. However, the widespread use of microalgae in agriculture is challenged by fragmented information and a lack of understanding of the impacts and mechanisms of microalgae in the soil and plants in different situations (Ibraheem, 2007; Metting and Rayburn, 1983; Rossi et al., 2017). Microalgae in the soil release bioregulators that promote the growth, including hormones, vitamins, carbohydrates, amino acids, and organic acids. These are the benefits and could be the source of abundance (Awale, 2017; Mfundo and Maqubela, 2012; Osman et al., 2010). Therefore, the objective of this study is to analyze the potential of microalgae as a source of nutrition in agriculture, as well as its health benefits.

MATERIALS AND METHODS

For this study, an experimental closed system (photobioreactor) was used for microalgae cultivation, because it is deemed to be the best method for obtaining biomass from them. Figure 1 shows the design of the photobioreactor that was used for the experiment.

Environment for microalgae cultivation

The first part of the experiment consisted on obtaining the first strain (the process was accelerated by fertilizing the cultivation environment, in this case river water and potable water with worm leachate); As a result of the photosynthetic action, the production of microalgae started in three to four weeks. The initial strain, river water, potable and

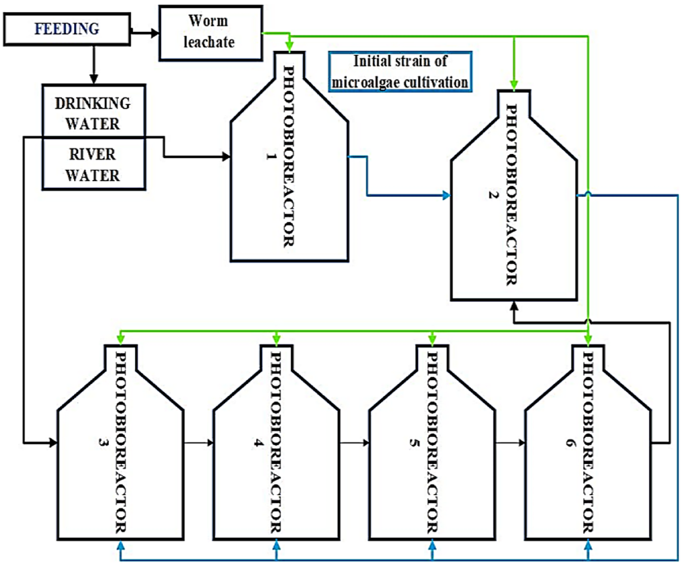

Figure 1. Photobioreactor diagram microalgae cultivation

worm leachate were used to multiply microalgae cultures, which slowly homogenize and produce microalgae after 7 days of photosynthetic activity. The process of sedimentation starts after 7 days, in order to extract the microalgae biomass. The process is repeated for each photobioreactor.

The control carried out on the cultures for better production of microalgae was at room temperature, since, above this, respiration increases, productivity is reduced, and it was not exposed to sunlight so that the cultures do not lose properties., since it is one of the main parameters that, due to photosynthetic action, is necessary for the development of biomass. On the other hand, the pH was ideal as consulted in the bibliography, because when changing it, the production of microalgae is paralyzed and can lead to degradation. Gentle stirring was carried out manually (for two minutes) to homogenize the mixture and incorporation of oxygen at least twice a day. The crops were fed every 8 days, the photosynthetic process being slower in the rainy season. Finally, resistance to environmental stress could occur if the physical and environmental conditions, such as pH, light intensity and temperature, were not ideal.

Characterization of physiochemical properties in microalgae cultures

Physical parameters

The temperature, pH, and conductivity were electrostatically (LAQUA portable tester measured to provide physical and the turbidity was measured using a turbidimeter nephelometer Sper Scientific 860040 (Morocho et al., 2023).

Chemical parameters

The following parameters were measured for the microalgae culture: ammonium, calcium, total phosphorus, phosphate, magnesium, nitrate, and total nitrogen. The ammonium was measured at 410 nm using a spectrophotometer and colorimetric technique (HACH Ammonia Nitrogen 10031). The magnesium was analyzed using an EDTA solution at pH 10, while the calcium was measured using a composite volumetric method at pH 12–13. The phosphorus was measured spectrophotometrically (HACH Total Phosphorus 10127) at 400–490 nm and nitrate at 220–275 nm Gomez (1987). The nitrogen content was calculated using nitrogen HACH 10071 through spectrophotometry. Phosphorus concentration was measured through spectroscopy of ascorbic acid (Lópes, 2019).

Drying the microalgae biomass

The microalgae biomass was dried using a digital scale BEL ITALINA ES1001 designed to adjust weight fluctuations every 20 minutes until the weight became constant. The heavy biomass was put in an aluminum tray and then put inside the oven at 50 °C for 6 h to eliminate the water from the biomass.

Characterization of physiochemical properties of microalgae dry biomass

According to AOAC (2005), the nitrogen (N) was measured using the Kjeldahl method, and the potassium (K) was measured with a flame photometer. Atomic absorption was used to measure the calcium (Ca), magnesium (Mg) and iron (Fe); the sodium (Na) was measured with a flame photometer; sulfur was measured again using a spectrophotometer at 420 nm. Phosphorus (P) was measured with a spectrophotometer, nitrogen (N) was analyzed using the Kjeldahl method, while potassium (K) was measured using a flame spectrophotometer. Calcium (Ca), magnesium (Mg), and iron (Fe) were measured by atomic absorption, sodium (Na) by flame spectroscopy, and sulfur (S) by a spectrophotometer at 420 nm. A spectrophotometer was used to quantify phosphorus (P) (Bray and Kurtz, 1945).

RESULTS AND DISCUSSION

Table 1 shows the results of the tests for each parameter during the maturation and/or fermentation stage of the culture every three days for each analysis. The results obtained vary, as certain numbers fluctuate while others remain constant. This is because during each characterization, the culture parameters were gradually increased while other parameters were reduced, with a delay of 3 days between each trial. Figure 2 shows the influence of time on the analyses.

Water is a key part of microalgae growth because it provides the nutrients (carbon, nitrogen, and phosphorus) needed for microalgae metabolism and reproduction (Lau et al., 1995). On the other hand, nutrients are necessary for the growth of organisms and microalgae. Nitrogen (N) is important for growth; it is absorbed as nitrate (NO_3) or ammonium (NH_4) and is necessary for the production of proteins, chlorophyll and cell structures (Khanzada, 2020).

In addition, these microorganisms require macronutrients including salt, calcium, and sulfate to grow, despite not carrying out vital physiological functions (Paskuliakova et al., 2018). These trace elements are iron, cobalt, magnesium, molybdenum, zinc and copper. The production of cellular components such as phospholipids, nucleotides and nucleic acids depends on phosphorus, a macronutrient essential for algae growth. According to Cerón Hernández et al. (2015), the adequate level of phosphate is 0.02–2 mg/l (Richmond, 2004).

The biomass content ranges from 0.05% to 3.3%, when microalgae cultures provide both ammonium and nitrate as nitrogen sources, nitrate is usually not used until all ammonium is depleted (Ullrich et al., 1990). The preference of ammonium over nitrate in microalgae is due to the energy needed by cells to convert nitrate into ammonium, unlike ammonium, which is a reducing compound that is directly incorporated into organic molecules such as amino acids (Bert et al., 1984; Ullrich et al., 1984). Nitrogen is the second most abundant element in the biomass of algae species. According to Richmond (2004), the nitrogen content is between 1% and 10% of dry mass.

In addition, microalgae require simple nutrients such as N, P, and K, as well as trace amounts of B, Cu, Mn, Mo, Co, V, and Se (Li et al., 2008). One of the most important nutrients for microalgae growth is nitrogen. Lack of N2 increases lipid

Table 1. Characterization of the physicochemical properties of microalgae cultures

Physical parameter	Test 1	Test 2	Test 3	Unit	Median	SD
Conductivity	480	492	502	us/cm	491.33	8.99
pH	8	9	9		8.67	0.47
Temperature	28.5	30,6	32	ºC	30.37	1.44
Turbidity	25.8	32,8	40,4	NTU	33	5.96
Chemical parameters						
Ammonium	0.5	0.4	0.5	mg/L	0.47	0.05
Calcium	48	48	48	mg/L	48	0
Total phosphorus	2.5	2.2	1.9	mg/L	2.2	0.24
Phosphate	0.05	0.07	0.06	mg/L	0.06	0.01
Magnesium	1.46	1.22	1.7	mg/L	1.46	0.20
Nitrate	8.2	9.4	10.7	mg/L	9.43	1.02
Total nitrogen	3.7	4.1	5	mg/L	4.27	0.54

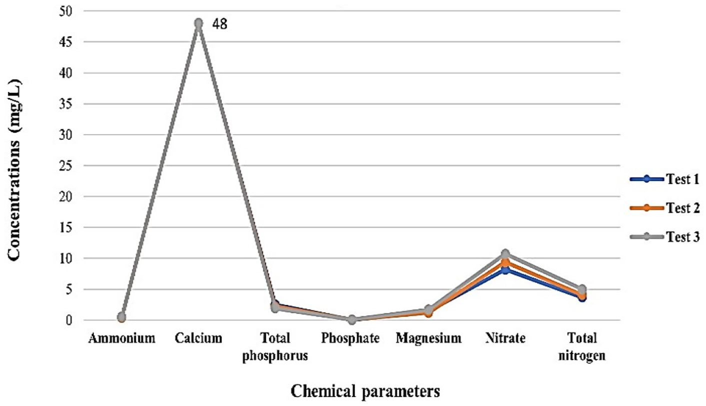

Figure 2. Characterization of the physicochemical parameters of microalgae cultures

levels by 20% to 40% in the algae environment (Abdel-Raouf et al., 2012; Park et al., 2011).

Table 2 shows a summary of the obtained results from drying the microalgae biomass and the moisture loss from the weight variation control every 20 minutes until the weight was constant, as it is shown in the Figures 3 and 4.

Figure 3 shows that moisture loss changes between 0.88 and 0.856, while the temperature and oven drying time result in a large moisture weight loss between 0.856–0.724.

Figure 4 shows some weight loss and deformation with moisture content, indicating that the drying rate as a function of moisture ranges from 0.059 to 0.015. We also see that for drying rates ranging from 0.231 to 0.006, water loss is greater and decreases over time. Weight loss decreased from 0.006 to 0.017 until moisture loss stabilized.

Table 2. Kinetic of drying

Initial drying temperature: 50 ºC				
Water mass		284.099 g		
Solid mass		38.741 g		
Relative moisture. Microalgae biomass: 88%				
Hours	Weight (g)	Base moisture dry (XBS)	Free moisture	∆x/∆t
0	322.84	0.88	0.156	0.017
0.2	313.8	0.876	0.152	0.014
0.4	306.54	0.873	0.149	0.005
1	299.31	0.870	0.146	0.017
1.2	291.33	0.867	0.143	0.018
1.4	283.31	0.863	0.139	0.005
2	276.42	0.859	0.136	0.021
2.2	268.14	0.855	0.131	0.231
2.4	203.09	0.809	0.085	0.014
3	194.15	0.800	0.076	0.036
3.2	187.29	0.793	0.069	0.052
3.4	178.26	0.782	0.058	0.015
4	171.04	0.773	0.049	0.038
4.2	165.48	0.765	0.042	0.052
4.4	158.4	0.755	0.031	0.018
5	151.56	0.744	0.020	0.044
5.2	146.43	0.735	0.011	0.058
5.4	140.21	0.723	0	0
6	140.21	0.723	0	0

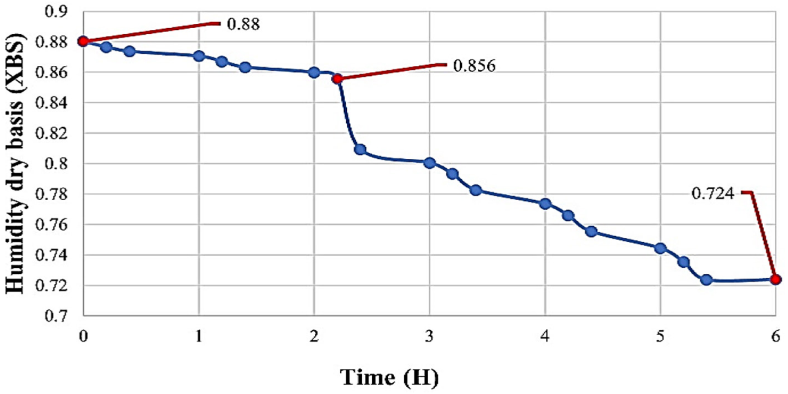

Figure 3. Drying kinetic curve

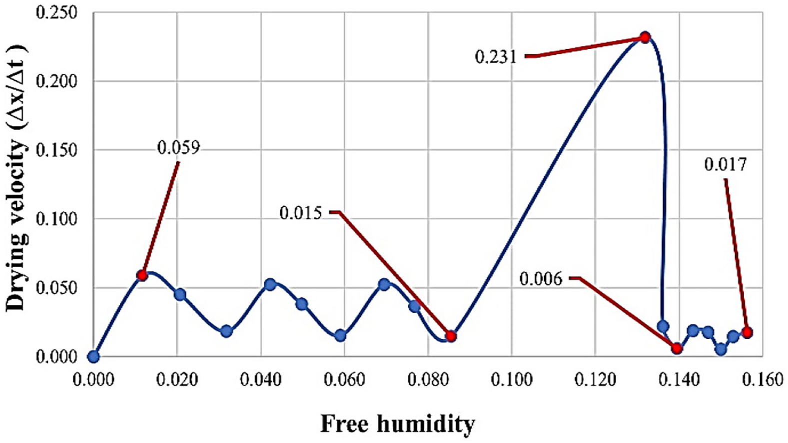

Figure 4. Drying velocity curve

The response zones obtained from the curves produce results in the graphics that result that fluctuate with temperature and time and, in certain situations, the percentage of moisture falls drastically.

In Table 3, the obtained results from the characterization of tests carried out to the dry microalgae biomass. In Table 3, the biofertilizers produced from microalgae are shown as possible sustainable alternatives, as well as economically valuable and respectful of the environment, in contrast with chemical fertilizers. They not only increase agricultural yields, but also improve the soil's physiochemical properties and reduce pollution.

Although the values of the macronutrients N, P, K were relatively low compared to the chemical fertilizer; However, the values of micronutrients such as: Ca, Mg, S, B, Zn, Cu, Fe and Mn, presented a higher percentage and concentration, which can help in soil fertility, and, above all, in Plants in their initial state also have a high fertilizing power, given that all the chemical elements are easily absorbed through the stomata or through the cuticle, which could partially replace the dose of chemical fertilizer, whose efficiency is usually limited. due to chemical and physical restrictions of the soil.

On the other hand, and despite a lower N–P–K content, seaweed fertilizers improved plant growth to a similar degree as chemical fertilizers. This is likely due to the higher amounts of other micronutrients that helped moderate the amounts of primary nutrients needed.

Additionally, Table 4 shows the results of the present work with other authors and a fertilizer with respect to the nutrients and micronutrients analyzed. In this sense, as mentioned above, obtaining biofertilizer from microalgae is presented as an important option for its application in agriculture. The variation in composition can be attributed to the way of feeding and the species established by the authors presented in the Table 4.

Comparison between microalgae biofertilizers and chemical biofertilizers

Algae biomass contain les nitrogen, phosphorus and potassium (N, P, K), the main components

Table 3. Characterization of dry biomass

Parameters	Test	Chemical fertilizer (Triple 15-15-15)	Units
Nitrogen (N)	11	12.4	%
Phosphorus (P)	1.4	6.6	%
Potassium (K)	0.3	12.5	%
Micronutrients			
Calcium (Ca)	0.69	0.1	%
Magnesium (Mg)	0.43	-	%
Sulfur (S)	0.15	-	%
Iron (Fe)	998	455	ppm
Copper (Cu)	9	-	ppm
Manganese (Mn)	91	26,1	ppm
Boron (B)	24	-	ppm
Zinc (Zn)	16	-	ppm

Table 4. Characterization of biofertilizers

Species	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Sulfur (S)	Iron (Fe)	Copper (Cu)	Manganese (Mn)	Boron (B)	Zinc (Zn)	References
	%						ppm					
	11	1.4	0.3	0.69	0.43	0.15	998	9	91	24	16	
B. Chlorella sp and *Scenedesmus* sp.	0.10	0.13	0.19	0.9	0.02	0.03	3.4	0.2	3.2	3.8	6.4	Gonzalo, 2020
B. Scenedesmus sp	1.74	0.24	1.77	3.15	0.85	0.21						López, 2022
B. Arthrospira spp.	6.70	2.47	1.14				12.4		11.5		292	Pérez-Madruga et al. 2020
B. Sprilunia plantesis	7.8	0.8	1.6	0.4			1057		41.9		155.4	Wuang et al., 2016
Chemical fertilizer (Triple 15-15-15)	12.4	6.6	12.5	0.1			455		26.1			Wuang et al., 2016

of these fertilizers. The primary nutrients often make a bigger contribution because crops absorb these nutrients with more frequency than secondary and micronutrients. With the exception of N; P; K, all the analyzed nutrients (calcium, iron, manganese, zinc y selenium) were present in great quantities in algae biomass. Calcium is not only found in cell walls, but is also important in the formation of new meristems and root tips. Calcium prevents meristems from becoming stiff and brittle by granting flexibility and stretchability to cell walls (Wuang et al., 2016).

The algae provided three times more calcium and twice as much iron as the artificial fertilizer. Many plants suffer from iron deficiency, as it is necessary for the production of several enzymes. Manganese is found in algae biomass at higher levels than in artificial fertilizers. Zinc is used in the formation of growth compounds and enzyme systems; however, it does not appear in fertilizers. Vitamin B6 is necessary for the creation of carbohydrates and chlorophyll, as well as to improve a variety of metabolic functions. Algae contain trace amounts of selenium, a mineral not found in fertilizers. Algae fertilizer promotes plant development at the same level as chemical fertilizers, but with less nitrogen, phosphorus, and potassium. This could be because the fertilizer contains additional secondary chemicals (Wuang et al., 2016).

These organic microalgae-based fertilizers are suitable for a variety of crops, including flowers, fruit trees, cereals, beans, vegetables, and onions. Much research has documented the use of dried cyanobacteria to improve rice soil fertility (Lavu et al., 2013; Watanabe et al., 1951). According to these findings, field trials often result in a 15 to 20% increase in rice yield. Previous research has only looked at the impacts of inoculating spirulina on rice, but the current situation indicates that it could be used for other crops in the future (Ronga et al., 2019). The introduction of microalgae in tomato crops (Scenedesmusquadricauda Chodato *Chlorella vulgaris* Beyerinck) had great results, as microalgae (*S. quadricauda*) improved the growth of tomato shoots and microalgae biomass. Adding dried chlorella powder to the soil can improve lettuce production (Das et al., 2019). In addition, certain compounds in biomass protect plants from disease.

In general terms, the use of biomass from microalgae is profitable, since several studies demonstrate this when applying it to different types of crops, which has some benefits capable of improving the physical and chemical properties of the soil and plants. or products that can be grown, such as: flowers, fruit trees, cereals, beans, vegetables and vegetables, being N, P, K and the micronutrients necessary for growth, improving the quality of the products and therefore health of people at the time of consuming them.

CONCLUSIONS

The use of microalgae biomass as a biofertilizer in agriculture can increase fertility, reduce soil erosion and nutrient loss, and improve soil quality over time. It also benefits plants, vegetables and vegetables, as it contains nitrogen, phosphorus and potassium, necessary for growth.

The quality of the microalgae culture water was within the acceptable parameters recommended by several authors, and its physicochemical properties were sufficient for a medium in which microalgae could grow in any concentration.

Microalgae are a good alternative for crops because they protect the health of both the producer and the consumer by generating pesticide-free products. As previously stated, this is due to its functional features, rapid development, and ease of manufacturing. This allows for further research and application of microalgae in a variety of industries, including biotechnology, cosmetics development, human and animal nutrition, etc.

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