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Evaluation of a vermicompost leachate as a mixotrophic culture medium for *Scenedesmus acutiformis* in a column photobioreactor with potential for biodiesel production

Evaluación de un lixiviado de vermicomposta como medio de cultivo mixotrófico para *Scenedesmus acutiformis* en un fotobiorreactor de columna con potencial para la producción de biodiesel

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Abstract

Currently, efforts are being made to establish low-cost and easily scalable cultivation systems, through the use of alternative culture media is studied, such as leachates from the degradation of organic material with a variety of nutrients that can be assimilated by microalgae. Therefore, in this research, the lipid yield of *Scenedesmus acutiformis* were evaluated in a column photobioreactor of 3 liters for 30 days, using as a medium cultivate a vermicompost leachate at a total nitrogen concentration of 0.5 mg L^{-1}, from the compost consisting residues of papaya, green grass, dry grass, almond leaves and bovine manure, with nitrogen concentrations of 0.23, 0.55, 0.93, 0.67 and 1.13%, respectively. Three concentrations (15, 25 and 50%) were prepared with two lighting levels (46.25 and 64.75 μmol photons m^{-1} s^{-1}) and photoperiod 12/12, were evaluated. The best treatment was leachate at 50%, produced 28.7% lipids. The profile of fatty acids showed linolenic acid as the most abundant (27.01%), followed by linoleic acid, palmitic acid, stearic acid, oleic acid, palmitoleic acid at concentrations of 17.05, 16.46, 14.70, 12.75 and 3.69%, respectively. The results obtained show the potential of vermicompost leachates standardized and characterized as a culture medium for the production of lipids.

Keywords: mixotrophic, leached, photobioreactor, photoperiod, biomass.

Resumen

Actualmente se están realizando esfuerzos para establecer sistemas de cultivo de bajo costo y fácilmente escalables, mediante el uso de medios de cultivo alternativos, como son los lixiviados provenientes de la degradación de materia orgánica con una variedad de nutrientes asimilables por las microalgas. Por lo tanto, en esta investigación, se evaluó el rendimiento lipídico de *Scenedesmus acutiformis* en un fotobiorreactor de columna de 3 litros durante 30 días, utilizando como medio de cultivo un lixiviado de lombricomposta a una concentración de nitrógeno total de 0,5 mg L^{-1}, proveniente del compost compuesto por residuos. de papaya, pasto verde, pasto seco, hojas de almendro y estiércol bovino, con concentraciones de nitrógeno de 0.23, 0,55, 0,93, 0,67 y 1,13%, respectivamente. Se evaluaron tres concentraciones (15, 25 y 50%) con dos niveles de iluminación (46.25 y 64.75 μmol fotones m^{-2} s^{-1}) y fotoperiodo 12/12. El mejor tratamiento fue el lixiviado al 50%, produciendo 28,7% de lípidos. El perfil de ácidos grasos mostró al ácido linolénico como el más abundante (27,01%), seguido del ácido linoleico, ácido palmítico, ácido esteárico, ácido oleico, ácido palmitoleico en concentraciones de 17,05, 16,46, 14,70, 12,75 y 3,69%, respectivamente. Los resultados obtenidos muestran el potencial de los lixiviados de lombricomposta estandarizados y caracterizados como medio de cultivo para la producción de lípidos.

Palabras clave: mixotrófico; lixiviado; fotobiorreactor, fotoperiodo, biomasa.

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1 Introduction

In recent years, the search for more resilient forms of food and energy production has intensified (Maroneze *et al*., 2021). In this sense, biomass from microalgae has been identified as a raw material with high potential, since it offers an important variety of possibilities in the generation of compounds of interest in the food, pharmaceutical, energy areas, among others (Wibisono *et al*., 2019). However, despite the advances in its cultivation, there are several challenges that do not allow its scaling to an industrial level, especially those related to the optimization of culture systems and media.

Microalgae are very abundant photosynthetic organisms in nature, which have autotrophic, heterotrophic and mixotrophic metabolism. This particularity can be used to modulate their cultivation conditions and thereby favor the metabolic production of lipids, carbohydrates, proteins, pigments, etc. (Barros de Medeiros *et al*., 2022). Given the variety of microalgae species that exist and the commercial importance they have achieved, it is required to identify optimal culture systems and media for specific microalgae strains, and for these culture systems to optimize the productivity of the metabolite of interest (May-Cua *et al*., 2019).

In addition to autotrophic and heterotrophic metabolism, microalgae can grow under mixotrophic conditions, using light as an energy source and organic carbon as carbon sources (Meng *et al*., 2020; Condiri *et al*., 2023). The most important quality of the mixotrophic culture is the ability of microalgae to simultaneously metabolize organic and inorganic carbon, through cellular respiration and photosynthesis, respectively, thus maintaining both autotrophic and heterotrophic metabolism (Zhan *et al*., 2017). Thus for microalgae to carry out mixotrophic metabolism, the presence of light is required for the biofixation of CO_2 through photosynthesis and organic substrates for aerobic respiration, while in the absence of light the metabolism becomes heterotrophic (Perez-Garcia and Bashan, 2015). This type of metabolism allows valorize the nutrients of agroindustrial residues and favors the use of alternative culture media such as leachates or wastewater, therefore, this substrate can be used as a medium of dual strategy crops, biomass production and bioremediation (Ye *et al*., 2020; Yousuf, 2020; Condiri *et al*., 2023). Vermicompost leachates are liquids obtained from the biological degradation of organic matter by worms, the most used being *Eisenia fetida*. Depending on their source, they have a varied composition of macro and micronutrients, however the metabolic capacity of microalgae allows them to grow in media with these characteristics, making vermicompost leachate a viable option for obtaining biomass and lipids that can be used for the production of biodiesel, as well as other high-density compounds value for the industry (Xin *et al*., 2010; Grabska *et al*., 2015; Serejo *et al*., 2020).

For the production of microalgal biomass, two main cultivation systems are available, open and closed. However, there is currently no system that adapts to all processes. Taking this aspect into account, current research is focused on the search for highly efficient systems with low operating costs (Hsieh-Lo *et al*., 2019; Assunção *et al*., 2020). In this sense, closed systems called photobioreactors (FBRs) are available, which, although they have deficiencies, offer significant advantages in the control of culture parameters, the diversification of materials for their construction, as well as the optimization of the system for morphotypes of specific microalgae. Column FBRs consist of a series of straight transparent plastic or glass tubes, which can be arranged in different patterns and with different orientations (Maroneze and Queiroz, 2018), the aeration of the system occurs through a bubble column or through airlift, driven by a pump that supplies air and at the same time facilitates gas exchange, the distribution of nutrients, in addition to avoiding the sedimentation of the biomass (Sirohi *et al*., 2022).

Another important aspect is the selection of microalgae species suitable for biodiesel production, not only the productivity of the biomass depends on this factor, but also the lipid yield and the profile of fatty acids accumulated by the cell, since the calorific value of the resulting biodiesel depends on this composition. Saturated and unsaturated fatty acids have been identified that contain between 12 and 22 carbon atoms, therefore, the group that best meets all these characteristics are the microalgae that correspond to the Chlorophyceae group (Mathimani *et al*., 2020; Chen *et al*., 2021).

According to the above, the objective of this research work was to evaluate the lipid productivity and fatty acid profile of the microalgae species *Scenedesmus acutiformis* using a vermicompost leachate in mixotrophic condition as a culture medium. The evaluation was carried out in an FBR of spine for 30 days.

2 Materials and methods

The experiment was established in the Universidad Politécnica de Chiapas, located in Suchiapa, Chiapas, México. The geographic location was latitude 16° 45’ 11" north and longitude 93°06’ 56", corresponding to tropical regions, with more than 1100 mm annual rainfall. During the experimental period the temperature was maintained to 27 to 30 ºC, the air relative humidity was maintained at 65-69%, with the objective of evaluating to *Scenedesmus acutiformis* in the lixiviated from vermicompost for the production of lipids destined for the production of biodiesel.

2.1 *Algal sample*

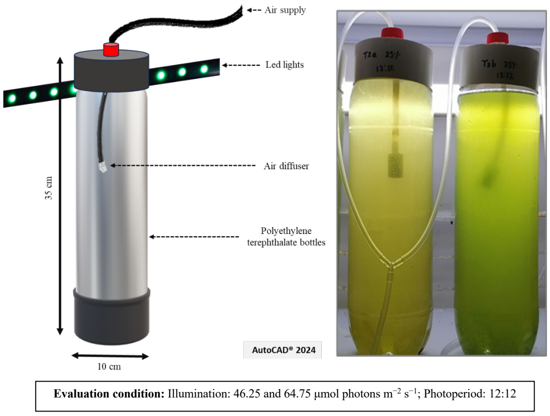
The algae sample *Scenedesmus acutiformis* belongs to the collection of cultures of the laboratory of the Universidad Politecnica de Chiapas, Mexico. The sample was observed with microscope for identification and determination the size frequency of algal cells per ml of suspension using a FlowCam (Fluid imaging Technologies), the sizes were expressed as equivalent length (EL) (Sieracki *et al*., 1998). The algal species was morphologically same as *Scenedesmus acutiformis*, according to Sheath and Wehr, (1973) and morphologically validated; the latin scientific name and class were confirmed in the database of AlgaeBase ID: 27859 (`http://www.algaebase.org/`); Dyntaxa ID: 257449 (`http://www.artdata.slu.se/dyntaxa`); REBECCA code: R0755 (`http://www.freshwaterecology.info`) and database of NCBI (`https://www.ncbi.nlm.nih.gov/pubmed/`).

2.2 *Obtaining vermicompost leachate as a mixotrophic culture medium*

First, the establishment of the compost was carried out to obtain the leachate, for this a mixture of waste was used that contained 35% of bovine manure, 5% of green grasses, 50% of dry grasses and almond leaves and 10% of papaya waste, a total of 60 kg of waste was composted. Composting was carried out for 60 days with 30 days of maturation. During this period, humidity, temperature and pH were monitored every day. Once maturation was reached the vermicomposting was carried out, adding 1Kg of Californian red worms (*Eisenia fetida*) to the compost, which allowed obtaining a leachate in accordance with the Mexican Standard (NMX-FF-109- SCFI-2007) "Worm humus (vermicompost) - specifications and test methods". The leachate was collected by gravity drip and stored at room temperature in plastic bottles with a capacity of 5 liters.

2.3 *Physicochemical characterization of the compost waste and vermicompost leachate*

The physicochemical characterization of the waste that made up the compost mixture was carried out, using as a reference the official Mexican standard NMX-FF-109-SCFI-2007 for the determinations of humidity, ash, organic matter (OM) and organic carbon (OC), considering NOM-021-RECNAT-2000 for the validation of the aforementioned determinations and the pH with the use of a potentiometer (HANNA HI 2211-01). Total nitrogen (N) was analyzed by Kjeldahl method (AOAC 2000; Li *et al*., 2015). phosphorus and potassium contents were analyzed by colorimetric analysis in a spectrophotometer (Shamim *et al*., 2015), finally the turbidity was determined according to Momeni *et al*. (2018).



Evaluation condition: Illumination: 46.25 and 64.75 μmol photons m^{-2} s^{-1}; Photoperiod: 12:12

Fig. 1. Column photobioreactor with a capacity of 3 L for production of microalgal biomass from *Scenedesmus acutiformis* in mixotrophic condition.

2.4 *Commissioning of the column photobioreactor*

For cultivation, a column photobioreactor (FBR) was used, which was built using polyethylene terephthalate bottles with a diameter of 10 cm and a height of 35 cm, with a capacity for 3 L. A hole was placed at the top to supply constant air flow, with the use of a 200-240V AKKEE brand fish tank air pump with an aeration of 1.4vvm (Fig. 1). A photoperiod of 12:12 was maintained, lighting was provided using 16 Watt LED lamps, which were placed around the FBR at a distance of 10 cm (Fig. 1). The leachate obtained was filtered with the help of a vacuum pump with Whatman number 1 filter paper of 0.45 μm and was incorporated and evaluated at three leachate concentrations (LIX) 15, 25 and 50% with two levels of light intensity (46.25 and 64.75 μmol photons m^{-2} s^{-1}), which allowed the evaluation of 6 treatments (T1: 46.25 μmol photons m^{-2} s^{-1} and 15% LIX; T2: 46.25 μmol photons m^{-2} s^{-1} and 25%LIX; T3: 46.25 μmol photons m^{-2} s^{-1} and 50%LIX; T4: 64.75 μmol photons m^{-2} s^{-1} and 15%LIX : T5: 64.75 μmol photons m^{-2} s^{-1} and 25%LIX; T6: 64.75 μmol photons m^{-2} $s^{-1}$and 50%LIX). The culture was started at a concentration of 1.3×10^3 Cell of *Scenedesmus acutiformis*, with an evaluation period of 30 days.

2.5 *Quantification of cell density and biomass productivity*

The concentration of microalgal biomass was determined in all treatments by daily cell counting using a Neubauer hemacytometer. The data generated were used to construct the growth curves, the specific growth rate (μ) and the doubling time (Td) in the exponential phase were determined according to Wood *et al.* (2005).

After 30 days of evaluation, the recovery of the biomass produced was carried out by simple filtration using 6 μm diameter filter paper. It was left to dry at room temperature for subsequent extraction of the oil.

2.6 *Oil extraction from Scenedesmus acutiformis*

To obtain lipids, the Soxhlet extraction technique was used with 2g of dry microalgae on the cellulose thimble cartridge (25x80 Whatman) as a filter, using 98% hexane as a solvent. The extraction was carried out for two hours at a temperature of 68 °C. Subsequently, it was distilled using a rotoevaporator to recover the oil. Finally, the round bottom flask was weighted and the percentage of oil extracted was obtained by difference in weight (Sánchez-Roque *et al.*, 2020).

2.7 *Fatty acid profile of total lipids of Scenedesmus acutiformis*

Fatty acid profile analysis wise pooled isolated from *Scenedesmus acutiformis* was carried out using gas chromatography mass spectroscopy (GC-MS). Fatty acid methyl esters were prepared using following procedure: 30 mg of total lipid dissolved in 1 ml of methanol was mixed with 1 ml of 12% solution of KOH prepared in methanol. To this solution equal volume of 5% HCl in methanol was added and heated at 75 ºC for 15 min. This solution was allowed to cool and 1 ml of distilled water was added and shaken. Upper organic layer containing fatty acid methyl esters (FAMEs) was carefully transferred to a new clean vial. GC-MS analysis of FAMEs was performed using diethylene glycol succinate capillary column (30m x 0.25 x 0.25 μm). 100 μl of methyl ester sample solution was injected for each analysis. Helium was used as a carrier gas. The injector temperature was 180 ºC and detector temperature was 230 ºC which was increased to 300 ºC at a temperature gradient of 15 ºCmin^{-1} (Härtig, 2008).

2.8 *Statistic analysis*

To determine significant differences between the treatments evaluated the one way ANOVA test was carried out at $p<0.05$ level of significance; the statistical software used was the STATGRAPHICS PLUS (1999) for windows. For the first experiment, prior to statistical analysis, data were assessed three replicate experiments, for equality of variance and normality. A Tukey test was carried out following to compare means of treatments.

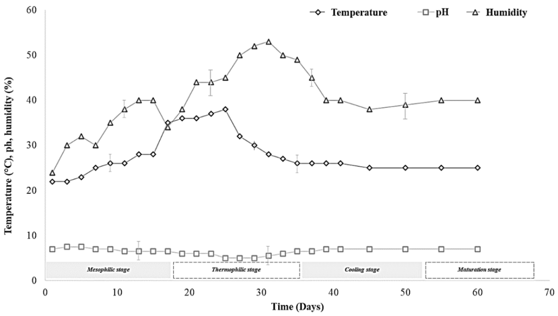


Fig. 2. Compost degradation kinetics over 60 days. The averages of three repetitions (± standard error) for each point.

3 Results and discussion

3.1 *Physicochemical characterization of the compost waste and vermicompost leachate*

The physicochemical characterization of the compost materials showed that there is greater humidity in fresh waste such as papaya (PA) and green grass (PV), while organic matter is higher in bovine manure (EB), followed by almond leaves (HA) and PV. Regarding organic carbon (OC) and total nitrogen (TN), it is observed that green grass is the one with the highest values with a C/N ratio of 10.81 (Table 1).

All the waste was placed in layers to form a compost and begin a 30-day degradation (Albalate-Ramírez *et al.*, 2023). In this sense, in the degradation kinetics of the waste (Fig. 2), it can be seen that until day 17 the mesophilic stage is maintained where the temperature reached is 35°C and the pH is 6, the thermophilic stage was observed from day 18 to 30, reaching a maximum temperature of 38°C. The cooling of the compost began on day 50 and subsequently a maturation period was maintained for 30 days. At the end of the compost degradation process, the worm humus had a pH of 7.5 and a humidity of 39% and a color from black to dark brown was observed, with the smell of moist earth, absent of putrid odors, the porosity of the humus is ≥ 5 mm.

The leachate, on the other hand, reached a pH of 7.85, where the most abundant nutrient is phosphorus with a concentration of 8.02 mg kg^{-1}. However, the content of nitrogen is limited with 0.5mg L^{-1} (Table 2), benefiting the accumulation of lipids in

Table 1. Physicochemical characterization of the materials that made up the compost.

Sample	%Humidity	%Ash	%OM	%OC	%TN	C/N
PA	92.43±1.71*	0.62±0.12	0.62±0.17	0.36±0.17	0.23±0.21	1.55±0.13
PV	71.90±1.50	12.43±0.08	12.43±0.08	7.21±0.08	0.55±2.76	13.04±0.72
PS	12.08±0.30	6.88±1.08	6.88±1.08	3.99±1.08	0.93±0.49	4.29±0.32
HA	10.20±0.06	14.68±0.75	14.68±0.75	8.51±0.75	0.67±0.35	12.8±0.13
EB	22.07±1.40	21.01±1.42	21.01±1.31	12.19±1.22	1.13±0.64	10.81±0.51

OC: Organic carbon; OM: Organic matter; C/N: Carbon nitrogen ratio; TN: Total nitrogen; PA: Papaya, PV: Green grass, PS: Dry grass, HA: Almond leaves, EB: Bovine manure. * Mean of three repetitions.

Table 2. Physicochemical characterization of vermicompost leachate.

Parameters	Concentration
Phosphorus	8.02 mg L^{-1}
pH	7.85
Total nitrogen	0.5 mg L^{-1}
Potassium	1.9 mg L^{-1}
Turbidity	3618 NTU

NTU: Nephelometric Turbidity Units; pH: Potential of hydrogen.

the microalgal biomass, such as it was demonstrated by Yang *et al.* (2014) by observing the varying $NaHCO_3$ and $NaH_2PO_4{\cdot}2H_2O$ concentration mutual interactions had a significant effect on the total lipid production value. The increase in $NaHCO_3$ and $NaH_2PO_4{\cdot}2H_2O$ concentrations enhanced the production of lipid initially, but then, with increasing their concentrations further, which exceed 3.07 and 15.49 mg L^{-1}, respectively, the lipid production decrease.

Likewise, an important value is pH, a variable that promotes establishing an optimal condition for the propagation of microalgal biomass, therefore, in previous works it has been determined that the best pH values are between 6 and 8, demonstrating the best activity metabolic by *Scenedesmus* sp. (Mandotra *et al.*, 2016; Waqar *et al.*, 2023), these values coincide with those obtained in the vermicompost leachate generated in this research work.

In this sense, knowing the waste that integrate the compost and its composition allow the standardization of the mixture and to know the physicochemical characteristics of the leachate that will guarantee metabolic acceptance by the microalgal biomass.

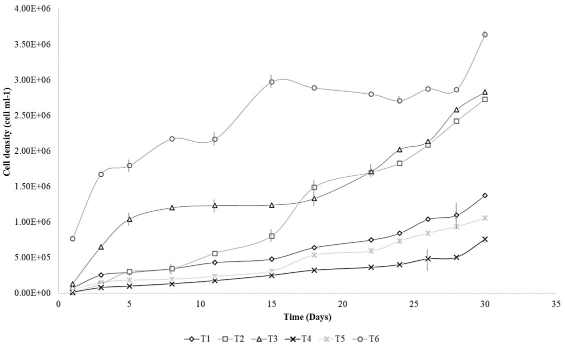


Fig. 3. Growth kinetics of *Scenedesmus acutiformis* in mixotrophic condition at FBR scale during an evaluation period of 30 days. The averages of three repetitions (± standard error) for each point.

3.2 Evaluation of the growth kinetics of Scenedesmus acutiformis in different concentrations of vermicompost leachate as a mixotrophic condition

It is important to mention that the variables that were constantly established in all treatments, such as photoperiod and aeration, are due to the fact that they correspond to an important factor that affects the growth of the genus Scenedesmus and biomass yield, considering the best conditions, the photoperiod 12/12 and aeration of 1.4 vvm, which will not allow the cells to remain static, quickly exhausting the substrate (Robles-Heredia *et al.*, 2016; Daneshvar *et al.*, 2021).

During the evaluation of the growth kinetics of *Scenedesmus acutiformis*, it was observed that the adaptation process of the microalgae began on the third day in all treatments and remained in the exponential stage until day 30, thereby reaching 6 logarithmic cycles (Fig. 3), demonstrating differences significant statistics between the concentration of the treatments evaluated (Table 3), even considering that the leachate was evaluated in crude form, without using antimicrobial treatments, these results are related by the chemical composition of the leachate and its capacity to assimilate carbon dioxide and nutrients. However, when large concentrations of nitrogen are present the competition between microalgae and nitrogen-oxidizing bacteria plays a significant role. Microalgae use nitrogen to synthesize lipids, proteins, photosynthetic pigments and nucleic acids, while nitrogen-oxidizing bacteria use it as a source of electrons and oxidize it to nitrite, considering that this leachate has low concentrations of nitrogen, it reduces competition with bacteria, thus also the initial microalgae concentration is high, to avoid competition

Table 3. Kinetic growth parameters and comparison of biomass productivity and lipid yield of *Scenedesmus acutiformis* in three different vermicompost leachate concentrations.

Leachate concentration %	Lighting level (μmol photons m^{-2} s^{-1})	Treatment	Initial cell density (cell mL^{-1})	Final cell density (cell mL^{-1})	Growth rate (Generations Day^{-1})	Generation of time (Day)	Dry biomass (mg L^{-1})	Lipid yield %
15	46.25	T1	$1.\times10^3\pm0.17^{a**}$	$1.4\times10^6\pm0.15^c$	0.113 ± 0.02^b	6.15 ± 0.03^c	65.15 ± 0.08^e	12.9 ± 2.01^d
	64.75	T4	$1.3\times10^3\pm0.12^a$	$7.6\times10^5\pm0.09^d$	0.202 ± 0.19^a	3.43 ± 0.11^d	31.08 ± 0.13^d	18.3 ± 0.01^c
25	46.25	T2	$1.3\times10^3\pm0.18^a$	$2.7\times10^6\pm0.03^b$	0.060 ± 0.07^c	11.50 ± 0.09^b	196.13 ± 0.17^b	12.7 ± 0.02^d
	64.75	T5	$1.3\times10^{3*}\pm0.11^a$	$1.1\times10^6\pm0.01^c$	0.060 ± 0.04^c	11.64 ± 0.05^b	111.73 ± 0.04^c	17.2 ± 0.04^c
50	46.25	T3	$1.3\times10^3\pm0.14^a$	$2.8\times10^6\pm0.07^b$	0.046 ± 0.08^c	15.15 ± 0.01^a	526.83 ± 0.09^a	25.3 ± 1.01^b
	64.75	T6	$1.3\times10^3\pm0.12^a$	$3.6\times10^6\pm0.16^a$	0.119 ± 0.06^b	5.81 ± 0.12^c	582.63 ± 0.16^a	28.7 ± 0.03^a

* Mean of three repetitions. **The averages (±standard error) within each column without common superscript differ significantly at $P < 0.05$.

for the substrate with other microorganisms, as reported by Gonzalez-Camejo *et al*. (2022); López-Alcántara *et al*., (2022) and Condori *et al*. (2023).

So, in Figure 3 it is observed that the treatments with the highest concentration of leachate obtained greater biomass productivity, with treatment T6 being the one with the highest productivity with a cell density of 3.6×10^6 cel mL^{-1} exposed to 64.75 μmol photons m^{-2} s^{-1} illumination, followed by the T3 treatment with a density of 2.8×10^6 Cel mL^{-1} exposed to 46.25 μmol photons m^{-2} s^{-1} illumination, both with a concentration of 50% leachate. However, the lowest yields are found in treatment T1 and T4 which contain a concentration of 15% of leachate (Table 3). In that sense, it has been proven mixotrophic production of *Scenedesmus* sp., using crude glycerol (CG) obtained from lab scale biodiesel unit in an internal loop airlift photo-bioreactor (ALR) for enhanced biomass and lutein production, observing that during the mixotrophic cultivation of *Scenedesmus* sp., in an ALR, the step-wise addition of CG during the growth phase improvement the productivity. Therefore, by increasing the concentration of the organic compound, the mixotrophic conditions for the microalga are potentiated, increasing the production of industrial metabolites of interest (Rajendran *et al*., 2020).

This is why mixotrophic growth is an alternative that is gaining interest due to its advantages, in which carbon sources are assimilated together with CO_2 (Patel *et al*., 2020). Due to the ability to assimilate carbon sources, these cultivations are less dependent on photosynthetic capacity (i. e. cell growth is not limited by light availability or affected by photoinhibition), which is reflected in higher biomass productivities (Li *et al*., 2020). On the other hand, unlike the heterotrophic growth, in mixotrophic cultivations, it is possible to produce light-induced metabolites, as it is the case under photoautotrophic cultivations (Sim *et al*., 2019; Castillo *et al*., 2021).

3.3 Evaluation of total lipid production

Regarding the productivity of dry biomass, the highest production occurred in the treatments with the highest concentration of leachate (50%) for treatments T3 and T6, with a productivity of 526.8 and 582.6 mg L^{-1} respectively. However, the lowest lipid production occurred in the treatments with a concentration of 15% leachate and the highest lipid productivity was 28.7% in treatment T6 with 50% leachate at 64.75 μmol photons m^{-2} s^{-1} of illumination, followed by treatment T3 with 25.5% lipids, with 50% leachate at 46.25 μmol photons m^{-2} s^{-1} illumination (Table 3).

These values are very similar to those obtained by Korozi *et al*. (2023) who under mixotrophic condition and under LED light illumination achieved a productivity of 26.6% of lipid accumulation of *Scenedesmus quadricauda*. Without a doubt, the nutrient content in the leachate as a culture medium was decisive for the productivity and accumulation of lipids, since the concentrations of nitrogen (N) and phosphorus (P) in the vermicompost leachate were limited, which has been demonstrated that algae of the phylum Chlorophyta can reach a lipid content between 20% and 30% of dry weight. Likewise, a study carried out by Kumari *et al*. (2021) reported a 10% reduction in *Chlorella vulgaris* biomass production and a 32% increase in lipids with 85% saturated fats under N and P limitation. It has been shown that the greatest accumulation of lipids is observed in the exponential phase of the microalgal biomass growth curve, when considering triacylglycerides as primary metabolites dependent on cell replication (Converti *et al*., 2009; Soto-León *et al*., 2014; Beltrán-Rocha *et al*., 2017), as seen in figure 3 in all treatments evaluated in this research were cultured after 30 days, when microalgal biomass continued to increase.

3.4 Fatty acid profile of total lipids of *Scenedesmus acutiformis*

The evaluation of the fatty acid profile of *Scenedesmus acutiformis* showed that 48.06% of the identified fatty acids correspond to polyunsaturated, 17.93% correspond to monounsaturated and 33.99% (Table 4 and Fig. 4), correspond to unsaturated fatty acids, as demonstrated by El-Sheekh *et al*. (2018) who carried out a screening of fatty acid profiles of morphotypes corresponding to different species of Scenedesmus, demonstrating an abundance of polyunsaturated fatty acids with 44.22%, 18.05% for monounsaturated fatty acids and 37.73% for saturated fatty acids, indicating that the balance in the composition of saturated and unsaturated fatty acids determines the good quality of biodiesel, reducing its oxidation and its viscosity

Table 4. Identification of the compounds present in the chromatogram of fatty acid profile of the oil obtained from *Scenedesmus acutiformis* propagated in 50% vermicompost leachate, as a mixotrophic condition intended for obtaining biodiesel.

N.	Compounds	Chemical formula	Parent ion (m/z)^2	RT%$^{-1}$ (min)	Fatty acid	Percentage of presence (%)
1	Linolenic acid	$C_{18}H_{30}O_2$	278,43	16.76	Polyunsaturated	27.01
2	Linoleic acid	$C_{18}H_{32}O_2$	280,44	18.57	Polyunsaturated	17.05
3	Palmitic acid	$C_{16}H_{32}O_2$	256,43	14.56	Saturated	16.46
4	Stearic acid	$C_{18}H_{36}O_2$	284,48	11.36	Saturated	14.70
5	Oleic acid	$C_{18}H_{34}O_2$	282.47	19.31	Monounsaturated	12.75
6	Palmitoleic acid	$C_{16}H_{30}O_2$	254,41	13.01	Monounsaturated	3.69
Saturated fatty acids (SFAS)						33.99
Monounsaturated fatty acids (MUFAS)						17.93
Polyunsaturated fatty acids (PUFAS)						48.06

^{1}RT: retention time, ^{2}Parent ion (m/z): molecular ions of the standard compounds (mass to charge ratio).

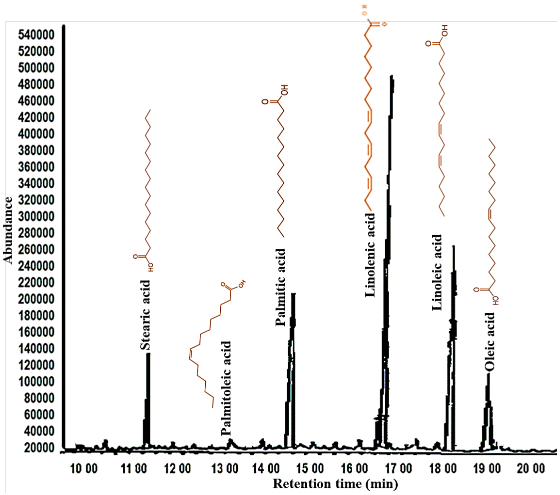


Fig. 4. Chromatogram of the fatty acid profile of the oil obtained from *Scenedesmus acutiformis* propagated in 50% vermicompost leachate, as a mixotrophic condition, using gas chromatography mass spectroscopy (GCMS).

and fluidity characteristics at low temperatures. The above, due to the structural features of fatty acids; such as chain length and degree of unsaturation; significantly influence the physical and chemical properties of biodiesel. The properties of biodiesel are determined mainly by its then Fatty Acid Methyl Esters (FAMEs) profile (Volgusheva *et al.*, 2022). The degree of unsaturation plays a significant role in fuel properties as the higher the degree of unsaturation of the FAMEs, the higher oxidation tendency of the biodiesel (Zhao *et al.*, 2019). On the other hand, shorter and more unsaturated fatty acids increase the viscosity and flow characteristics at low temperatures, which are undesirable characteristics. Therefore, a proper ratio between saturated and unsaturated fatty acids should be maintained in order to obtain biodiesel with appropriate characteristics (Tripathi *et al.*, 2021).

The present results revealed that Saturated fatty acids (SFAs) and Monounsaturated fatty acids (MUFAs) content of *S. acutiformis* was 33.99 and 17.93 % of total fatty acids, respectively (Table 4 and Fig 4), which would fulfill with the European standard specifications EN14214 and would result in oxidative stability of the biodiesel (Vignesh *et al.*, 2021). In addition, the conversion of triglycerides into FAMEs through the transesterification process would reduce the viscosity. Finally, one of the most important features of biodiesel is the carbon (C), indicates the longer the fatty acid carbon chains, with more saturated molecules present in the obtained biodiesel (Kokkinos *et al.*, 2015). It is for all the above that the lipids obtained from the biomass of *S. acutiformis* represent an alternative for the production of quality fatty acid methyl esters.

Conclusions

A vermicompost leachate was obtained as a culture medium for *Scenedesmus acutiformis*, where the percentage of nitrogen is limited (0.5mg L^{-1}), promoting lipid accumulation, demonstrating the ability to store up to 28.7% in a tubular photobioreactor of 3L, with a leachate concentration of 50% at 64.75 μmol photons m^{-2} s^{-1} with a 12:12 photoperiod, demonstrating a significant statistical difference in relation to the leachate concentration but not in relation to the light intensity.

This studied specie showed a highest significant lipid productivity with predominance of SFAs especially palmitic acid and MUFAs represented by oleic acid as the most abundant corresponding to a favorably properties how oxidative stability of the biodiesel. Therefore, the present study suggests *Scenedesmus acutiformis* as an attractive alternative renewable feedstock for biodiesel production.

For this reason, this work demonstrated the possibility of promoting the production of lipids with potential for biodiesel through the controlled production of vermicompost leachate as a culture medium.

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Abbreviations

TAG	triacylglycerides
FAME	methyl esters of fatty acids
GC-MS	gas chromatography mass spectroscopy
NTU	nephelometric Turbidity Units
OC	organic carbon
OM	organic matter
C/N	carbon nitrogen ratio
TS	total solids
LIX 15	Lixiviate at 15%
LIX 25	Lixiviate at 25%
LIX 50	Lixiviate at 50%
SFAs	Saturated fatty acids
MUFAs	Monounsaturated fatty acids
PUFAs	Polyunsaturated fatty acids

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