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Prototype flat photobioreactor with a settler for the cultivation of *Tetradesmus dimorphus* under mixotrophic metabolism under ambient conditions

Prototipo de fotobiorreactor plano con sedimentador para el cultivo de *Tetradesmus dimorphus* bajo metabolismo mixotrófico en condiciones ambientales

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Abstract

Large-scale biomass production is essential for the utilization of its metabolites. Closed and automated cultivation systems have many advantages, including better cultivation efficiencies, lower risk of contamination, and control of growth variables; however, their operating costs are greater than those of open systems, which generally use sterile culture and artificial lighting. To offer a cultivation alternative that combines the benefits of a closed system, under environmental conditions, a low-cost culture medium for mixotrophic metabolism and harvest by gravitational sedimentation, a prototype of a flat photobioreactor with a settler manufactured with common materials, the *Tetradesmus dimorphus* strain, was grown in previously disinfected eutrophicated lagoon water under natural conditions of radiation and temperature for 15 days. A productivity of 0.016 g/L/day was obtained, the harvest yield was 76 ± 0.95 % in 24 hours, and 23.6 % nitrate and 7.2 % COD were removed. Fourier transform infrared spectroscopy (FTIR) analysis of the produced biomass confirmed the presence of lipids, carbohydrates and proteins, while the extracted oils contained phospholipids and carboxylic acids.
Keywords: Microalgae, *Tetradesmus dimorphus*, photobioreactor, flat panel, gravitational sedimentation.

Resumen

La producción de biomasa a gran escala es fundamental para la utilización de sus metabolitos. Los sistemas de cultivo cerrados y automatizados tienen muchas ventajas, entre ellas: mejores eficiencias de cultivo, menor riesgo de contaminación, control sobre las variables de crecimiento, sin embargo, sus costos operativos son mayores en comparación con los sistemas abiertos, generalmente utilizan medios de cultivo estériles e iluminación artificial. Con el objetivo de ofrecer una alternativa de cultivo que combine los beneficios de un sistema cerrado, en condiciones ambientales, medio de cultivo de bajo costo para metabolismo mixotrófico y cosecha por sedimentación gravitacional, se propone un prototipo de fotobiorreactor plano con sedimentador, fabricado con materiales comunes, la cepa *Tetradesmus dimorphus* se cultivó en agua de laguna eutrofizada previamente desinfectada bajo condiciones naturales de radiación y temperatura durante 15 días, se obtuvo una productividad de 0,016 g/L/día, un rendimiento de cosecha 76 ± 0.95 % en 24 horas, una remoción de 23.6 % nitratos y 7.2 % DQO, el análisis de espectroscopía infrarroja por transformada de Fourier (FTIR) de la biomasa producida confirma la presencia de lípidos, carbohidratos y proteínas, mientras los aceites extraídos presentan fosfolípidos y ácidos carboxílicos.
Palabras clave: Microalga, *Tetradesmus dimorphus*, fotobiorreactor, panel plano, sedimentación gravitacional.

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1 Introduction

Microalgae are unicellular microorganisms rich in proteins and pigments of high value in the food industry, in addition to accumulating lipids and carbohydrates that can be used for biofuel production (Srivastava, 2019). However, to make bioconversion processes from microalgae viable, it is necessary to improve cultivation systems and cultivation efficiency, lower the cost of lighting, and reduce the energy cost of harvesting and the production process, i.e., develop sustainable and cost-effective processes.

Cultivation systems are divided into open systems called open ponds and closed or photobioreactors, such as flat panels, tubes, bubble columns, and bag reactors (Huang *et al*., 2017; Raheem *et al*., 2018).

Open systems are known to have ease of operation and low cost; however, they present risks of contamination and excessive evaporation of the culture medium and depend on climatic conditions (Ramos *et al*., 2018; Uma *et al*., 2014). For instance, the Mexican company Nanomex espirulina, located in Tlaxcala, cultivates this species in open ponds inside greenhouses under the control of temperature, relative humidity, and light (Peña *et al*., 2023).

Closed systems called photobioreactors allow the controlled operation of all variables and present a lower risk of contamination, require smaller areas per unit volume, have higher biomass productivity, and operate with natural or artificial light; additionally, cultures can develop under autotrophic or heterotrophic metabolism, and their operating costs are higher than those of open systems (Ahmad *et al*., 2021; Fernández, 2017; Lam *et al*., 2018).

On the other hand, the use of polluted natural waters and natural light in biomass production are alternatives for reducing pollution and biomass production costs. However, microalgal growth under natural lighting is more difficult due to variations in the availability and intensity of sunlight and temperature (Chen *et al*., 2016), fluctuations in the quantity of radiation, and differences in the quality of light, in addition to reaching reactors under direct and diffuse light (Leonardi *et al*., 2021), which is why the evaluation of biomass productivity under ambient conditions is necessary.

A fundamental step in obtaining microalgal biomass is harvesting, which generally consists of three steps. The first step is sedimentation, and the second step involves separating the biomass from the culture medium and concentrating it by filtration and centrifugation (Enamala *et al*., 2018; Faried *et al*., 2017). This process is challenging due to the microscopic size of the algae, which is estimated to represent between 20 and 30 % of the total cost of biomass production; therefore, this stage must be economically viable for commercial exploitation (May-Cua *et al*., 2019). Among the techniques used for harvesting are centrifugation, which can reach efficiencies of 80 to 90 % in minutes (Rashid *et al*., 2013); however, this requires energy. Other biomass harvesting techniques include flocculation and electrolysis, ultrafiltration (0.1 to 0.01 mm), and microfiltration (0.1 to 3 mm). Gravitational sedimentation has the advantage of not altering the chemical composition of the biomass, in addition to being the most economical; however, the yields are lower than those of other techniques such as centrifugation and filtration. On the other hand, for the production of third-generation biofuels, it is also necessary to reduce the energy consumption of the harvesting process.

The research and development of new models of photobioreactors that combine gravitational sedimentation of the crop, the use of natural light, and ease of sterilization between crops will allow progress in the production of biomass at low cost. The objective of this research is to develop a flat-layer photobioreactor with sedimentation and evaluate its performance by culturing *Tetradesmus dimorphus* under ambient conditions of radiation and temperature, as well as the gravitational sedimentation rate.

2 Materials and methods

2.1 Design

The proposed photobioreactor (FBR) is a closed system that allows the cultivation parameters to be controlled. This model consists of a quadrangular prism 50 cm wide by 48 cm high by 5 cm deep attached to a triangular prism 50 cm base by 20 cm high, as shown in Figure 1. Filling was performed at 38 cm height on a quadrangular prism. The surface area-to-volume ratio was calculated with Equation 1, the effective surface area of the FBR was calculated with Equation 2, only one side of the FBR was considered for illumination, the effective volume was determined with Equation 3, and finally, the S/V ratio was calculated with Equation 4. Approximate measurements have been used in the fabrication of flat photobioreactors, 45 by 48 cm, with different side lengths of 1.9, 3.8, and 7.6 cm depending on their cultivation volume (5, 10, or 20 L) (Wang *et al*., 2013).

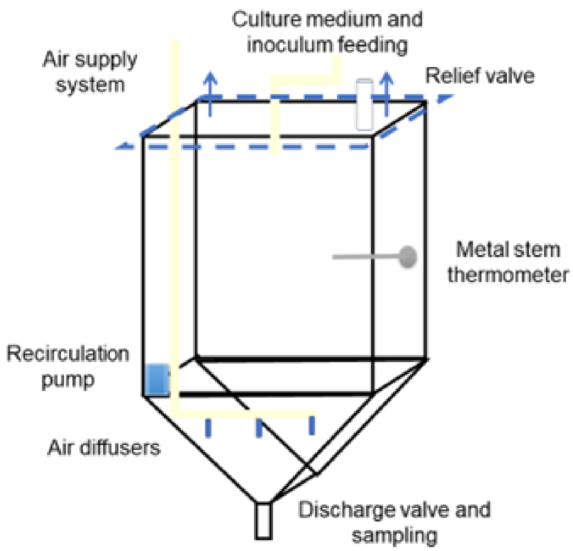

Figure 1. Flat-layer FBR with a settler.

$$Ratio\ \frac{S}{V} = \frac{Side\ plate\ area}{Equipment\ volume} \quad (1)$$

$$S = 0.5m * 0.38m + \frac{0.5m * 0.2m}{2} = 0.24\ \text{m}^2 \quad (2)$$

$$V = 0.05m * 0.5m * 0.38m + \frac{0.5m * 0.2m}{2} * 0.05$$
$$= 0.012\ \text{m}^3 \quad (3)$$

$$\frac{S}{V} = \frac{0.24m^2}{0.012m^3} = 20.0\ \text{m}^{-1} \quad (4)$$

The S/V ratio is the ratio between the illuminated surface area and the volume; the higher the S/V ratio is, the greater the cell concentration (Galvan *et al.*, 2014). The ratio recommended by (Grobbelaar, 2013; Rubio *et al.*, 2013) ranges from 20 to 200 m^{-1}. Therefore, the proposed FBR has the minimum recommended S/V ratio; on the other hand, the proposed geometry allows sedimentation of the cultured biomass. The effective volume of the FBR was 12 L, which is suitable for pretreatment and batch storage of lagoon water as a culture medium. The effective culture surface area of the FBR will be 0.24 m^2. To maintain a balance between the fed air and the O_2 produced in the photosynthesis stage of microalgae, it is necessary to allocate space in the FBR for gas exchange since in the photorespiration stage, the produced O_2 is consumed to generate CO_2 (Wang *et al.*, 2012). Therefore, the FBR was designed with a total volume of 14.5 L. To prevent excessive gas accumulation, a gas relief valve was installed.

Plexiglas (6 mm thick polymethylmethacrylate panel (PMMA)) was selected as the fabrication material for the construction of FBRs and is widely used as a substitute for glass because it provides high transparency, durability, chemical resistance, and low brittleness (Ashok *et al.*, 2019; Chen *et al.*, 2016; Feng *et al.*, 2011; Paladino and Neviani, 2021). Cylindrical glass containers (Kudahettige *et al.*, 2018) and polyethylene bags (Xu *et al.*, 2015) have also been used. Polyethylene and glass are fragile materials with a tendency to break, which would have made it impossible to disinfect the FBR between culture batches, so the FBR was manufactured in PMMA. For the loading of the culture medium and inoculum, 2.5 cm diameter polyvinyl chloride (PVC) tubing was installed, which was removed from the FBR with a 90° elbow, a 40 cm extension, and another 90° upward elbow with an 8 cm extension and threaded layer plug. To feed the FBR and close the system, all inoculations were always performed inside a biosafety cabinet (Labconco) in a sterile environment to prevent contamination of the culture.

The bottom of the FBR was designed in a V-shape to allow gravity settling and discharge, and a valve was installed for crop discharge. In the body of the FBR at half the height, a metal stem thermometer was installed on one side at a height of 25 cm to monitor the temperature of the culture. Microalgal cultures with mixotrophic metabolism consume organic and inorganic carbon. In this project, organic carbon is provided by the culture medium, while inorganic carbon is provided by an air feed with an atmospheric CO_2 content of 0.036 mg/L. Aeration is provided by two aquarium pumps with a flow rate of 4 L/min each; these pumps are coupled to the air pipe, and these types of devices have already been used in other microalgal culture FBRs (De Souza *et al.*, 2021). The air supply is filtered through sterile 0.45 μm syringe filters and distributed through chlorinated polyvinyl chloride (CPVC) tubing with three 2.5 cm long gas distributors to avoid sedimentation of particles at the bottom of the running FBR and to allow agitation of the culture. The selected air distributors were also used in the operation of other photobioreactors in previous research (Xu *et al.*, 2015).

To clean the FBR between batches, the top cover, indicated by the dotted blue line in Figure 1, must be removed to allow manual washing and resealing.

2.2 *Strain*

The *Tetradesmus dimorphus* strain was obtained from the phycology laboratory of the Universidad Autónoma Metropolitana Iztapalapa.

2.3 *Culture medium*

Eutrophicated lagoon water taken from the municipality of Altamira, Tamaulipas, Mexico, was used as the culture medium, with coordinates of 22.41881 latitude and -97.87649 longitude. The water was previously treated in an autoclave at 120 °C for 5 min, after which chemical oxygen demand (COD) was measured by the open reflux method recommended by NMX-AA-030/1-SCFE-2012 (Secretaría de Economía, 2012). In addition, nitrates and phosphates were measured with Hanna Instruments measurement kits (models H1781 and H1713).

2.4 Culture conditions

The FBR operation was performed in batch mode, with 20 % inoculum and 80 % culture medium, under natural conditions of radiation, temperature, and photoperiod for 15 days. Discontamination of the cultures was performed with a 5 % sodium hypochlorite aqueous solution (Cloralex) at a rate of 0.14 mL/L for 24 h with agitation, followed by neutralization with 24.81 % sodium thiosulfate at a rate of 0.1 mL/L (González, 2018), prepared with 99.5 % pure $Na_2S_2O_3$ (Fermont).

The temperature of the crop and the radiation (lux) received were recorded daily with a portable lux meter, model AS803 (Smart).

2.5 Growth

Biomass productivity was determined with Equation 5 (Ayatollahi *et al*., 2020), where X_f and X_i correspond to the dry biomass concentration in g/L at times final t_f and times initial t_i,

$$P = \frac{X_f - X_i}{t_f - t_i} \quad (5)$$

The dry biomass was determined gravimetrically with a 20 mL aliquot on 45 μm filters of known weight dried in an oven until constant weight.

2.6 Harvest

The biomass was left to settle for 24 h and then centrifuged at 6000 rpm for 15 min in an EBA 21 centrifuge (Hettich brand), after which the supernatant was removed. Subsequently, the biomass was lyophilized. The freeze-drying of the samples was carried out in 50 mL Falcon tubes covered with aluminum foil with 5 holes. The process was carried out at -80 °C for 2 days, after which the samples were placed in FreeZone 2.5 L Benchtop Freeze Dryers (Labconco). This method of harvesting was used in a previous investigation (Pérez *et al*., 2023).

2.7 Sedimentation performance

At the end of the culture period of each of the experiments, the air pumps and the culture medium recirculation pump were turned off to allow sedimentation of the cells for 24 h. The sedimentation efficiency was calculated based on the working volume and final concentration (g/L) measured in 2.5. The 100 % biomass was estimated based on the working volume and the final concentration (g/L) measured in section 2.5, and the corresponding percentage of harvested and freeze-dried biomass was calculated mathematically.

2.8 Nutrient removal

The initial and final concentrations of nitrates, phosphates and COD were used to determine the percentage removal, which was calculated with Equation 6 (Rosales *et al*., 2018).

$$\%\,Removal = \frac{Initial\ concentration - Final\ concentration}{Initial\ concentration} * 100 \quad (6)$$

2.9 Extraction of oils

Ultrasound-assisted lipid extraction with chloroform methanol has high yields (Pérez et al., 2023). Ultrasound-assisted extraction was performed at a 20 mL:g ratio and 20 % amplitude for 50 min at room temperature in a UP200Ht ultrasonic processor (Hielscher). The solvent was chloroform:methanol at a ratio of 2:1 V:V. This process was repeated three times, followed by filtration and evaporation of the solvent (Mamo and Mekonnen, 2020; Nguyen *et al*., 2019; Xaaldi Kalhor *et al*., 2016). Solvent evaporation was performed in a Büchi rotary evaporator (model R-134).

For the purification of the microalgae oil, 3 mL of H_2SO_4 was added to the sample to remove the chlorophyll, followed by centrifugation. The supernatant containing the chlorophyll was removed, and the sample was washed twice with 5 mL of hexane and heated at 90 °C for 15 min. After cooling, the sample was centrifuged, and the solvent was allowed to evaporate from the supernatant to obtain the lipids (Sacristán *et al*., 2014). All the reagents were 99.9 % pure (Fermont).

2.10 Biomass characterization

Infrared (FTIR) spectra of the biomass and oil of *Tetradesmus dimorphus* were obtained with a Fourier transform spectrometer (model spectrum 100, PerkinElmer brand), and the measurements were performed in the mid-infrared region in the range of wavelengths between 450-4000 cm^{-1} and 12 scans to obtain the spectra. FTIR spectroscopy is a tool used in previous research (Pérez *et al*., 2023) to identify functional groups belonging to microalgal metabolites.

Table 1. Environmental conditions and results of *Tetradesmus dimorphus* cultures in FBR.

Experiment	$C_{inoculum}$	Temperature	Lux	$C_{initial}$ (g/L)	C_{final} (g/L)	Productivity (g/L d)	Biomass obtained	% Harvest
1	0.24	28.92	1453.00	0.225	0.465	0.016	2.25	75.1
2	0.29	27.16	1458.62	0.275	0.485	0.014	2.03	77.0
3	0.34	26.80	1934.66	0.327	0.612	0.019	2.64	75.9

Table 2. Nutrient removal by *Tetradesmus dimorphus* in FBR.

Exp	NO^{-}_{3I}	PO^{-3}_{4I}	DQO_I	NO^{-}_{3F}	PO^{-3}_{4F}	DQO_F	% Removal NO^{-}_{3}	% Removal PO^{-3}_{4}	% Removal DQO
1	22.7	0.9	124.8	17.5	0.9	115.2	22.9	0	7.7
2	31.7	0.9	115.2	25.3	0.9	107.5	20.2	0	6.7
3	41.6	0.9	86.4	30.0	0.9	80.0	27.9	0	7.4

3 Results and discussion

Table 1 reports the results obtained from three cultures performed in the FBR, and Table 2 shows the initial (I) and final (F) values of nitrate, phosphate and chemical oxygen demand concentrations, as well as the percentage of pollutant removal.

Flat-plate FBRs provide a large illumination surface area, good light availability, good paths, relatively high biomass productivity, and low dissolved oxygen accumulation (Fazal *et al.*, 2018); compared with cylindrical FBRs, flat-plate FBRs also have a S/V ratio that allows efficient light transmission, which is characterized by a larger irradiance surface area.

The fabrication materials of the proposed FBR have already been used in other designs with different geometries for the cultivation of different species of microalgae. Paladino *et al.* (Paladino and Neviani, 2021) designed an airlift FBR with a 42 L total capacity, which was made of polymethyl methacrylate, polyvinyl chloride tubes, a perforated stainless steel plate, and a diffuser sponge to cultivate *Chlorella vulgaris* in ambient conditions with wastewater, and biomass productivity was not reported. *Chlorella vulgaris* was grown outdoors in a 50 L tubular FBR in nitrogen-reduced basal medium (1/4 nitrogen source) supplemented with 2 % CO_2 at a 0.05 vvm ratio, resulting in biomass productivities ranging from 0.037 to 0.045 g/Ld (Tran *et al.*, 2013). On the other hand, it was cultivated in a 50 L vertical tubular photobioreactor manufactured in polymethyl methacrylate, 50 L capacity, with an inoculum of 0.2 g/L, Bassal medium modified with 18.6 mM urea, and aeration of 0.05 vvm. Under ambient conditions, the biomass productivity was 0.26 g/Ld, and semicontinuous operation with a 50 % replacement ratio reached a productivity of 0.125 g/Ld (Chen *et al.*, 2016). On the other hand, *Chlorella minutissima* was cultivated in a tubular FBR with a useful volume of 18 L, manufactured with three acrylic tubes of 13.5 m inner diameter, 120 cm in height and a wall thickness of 5 mm, using Guillard F/2 culture medium at 25 °C with a constant luminosity of 15 lux, obtaining productivities from 0.023 to 0.074 g/Ld (Pereira *et al.*, 2018). Recent research proposed a 1.5 L cylindrical-conical FBR made of polymethyl methacrylate, with air feeding with an aquarium pump previously bubbled in distilled water before feeding, with a discharge valve for the clarified medium and a valve for the settled biomass, equipped with 24 W fluorescent lamps to control the photoperiod (May-Cua *et al.*, 2019).

A flat-panel FBR with a 6 L working volume made of glass 35 cm high, 25 cm wide and 10 cm in length, covered with an infrared blocking film, and operated with seawater at 25 °C, 1.5 L/min air, 12:12 L:D photoperiod, and 143.93 μmol photons/m2s allowed a biomass productivity of 0.773 g/Ld of *Nannochloropsis sp* (Nwoba *et al.*, 2019).

Nannochloropsis oceanica has been grown in a 60 L flat panel FBR with seawater under ambient conditions, where daytime temperatures above 40 °C cause the culture temperature to range from 20 to 30 °C despite the presence of internal heat exchangers in the FBR; however, biomass productivity has not been reported (Wang *et al.*, 2016). On the other hand, *Nannochloropsis gaditana* was grown in a set of three flat panel FBRs made of polyethylene that were 1.7 m high and 2.5 m long, with a metal base, and the spacing between each of them was 1. 5 m, covered by a leftover net that attenuated 90 % of solar radiation, operated in continuous mode with a dilution rate of 0.35 L/d in a culture medium with 10.0 mM NO_3 and 0.8 mM PO_4, produced 0.19 g/Ld of biomass (San Pedro *et al.*, 2016). These investigations did not report their harvesting procedures. Huang *et al.* (Huang et al., 2021) reported that microalgal growth is influenced by FBR dimensions, design, climate, and microalgal species.

On the other hand, a flat panel FBR made of 6 mm thick glass with dimensions of 60 x 60 x 5 cm in height, width and optical path, a working volume of 15 L and a total volume of 18 L was validated with the growth of *Chlorella vulgaris* in BBM with continuous lighting supplied with 4 LED lamps of 36 W and air 8 L/min for 8 days. At a density of 0.116 g/Ld of biomass (Martínez Roldan, *et al.*, 2020), the FBR proposed in this research presents a lower biomass productivity, which is attributed to the lower radiation and natural photoperiod; however, it favors biomass harvesting.

The preference for artificial culture media was observed, as was the investigation of municipal and industrial wastewater as culture media. The use of eutrophicated lagoon water in the culture of *Tetradesmus dimorphus* was evaluated in another study, where it was determined that an increase in light hours during the photoperiod decreased biomass productivity, and the optimal photoperiod was 10.5:13.5 L:D, with a productivity of 0.053±0.0015 g/Lday and 95.6 % COD removal in 33 days (Pérez *et al.*, 2023). On the other hand, vermicompost leachate has been investigated as a low-cost mixotrophic culture medium for the spice *Scenedesmus acutiforms*, reporting a growth capacity of 3.6 x 10^6 cells/ml when exposed to radiation of 64. 5 μmol/m^2, 12:12 photoperiod, 1.4 vvm aeration and 50 % leachate concentration (Zenteno *et al.*, 2024). The use of eutrophicated lagoon water as a culture medium is an alternative for reducing biomass production costs.

The 15-day culture time was not sufficient for phosphate removal; only 23.66 ± 3.90 % of the nitrates and 7.26 ± 0.51 % of the COD were degraded. At the end of 15 days, the culture medium still contained usable nutrients for microalgae; however, cultures that were not grown for longer periods (Borja *et al.*, 2017) reported superior removal efficiencies in *Chlorella vulgaris* culture, 54.22 % total phosphorus, 27.45 % total nitrogen and 49.68 % COD for 48 h, in wastewater and an airlift FBR of 7 L. Sandoval *et al.* (Sandoval *et al.*, 2020) reported that airlif bioreactors have advantages over stirred tank or bubble column reactors because they reduce cell damage and improve mass transfer and aeration.

The average biomass productivity obtained was 0.016 ± 0.002 g/L day, a value higher than that reported by (Chandra and Kalita, 2011) when growing *Tetradesmus dimorphus* in BG-11 medium supplemented with 0.1 g/L urea, which was 0.0015 g/L day, under lighting conditions of 2500 to 3500 lux, with a photoperiod of 16:8 L:D in 11 days of culture at 25 °C. (Laraib *et al.*, 2021) obtained 0.096 g/L day in BG-11 with a 12:12 L:D photoperiod and the addition of air at 11 L/min for 20 days; however, they increased productivity by supplementing the culture with 2 % P/V apple pomace hydrolysate at 0.14 g/L day, i.e., it presented greater biomass productivity in mixotrophic

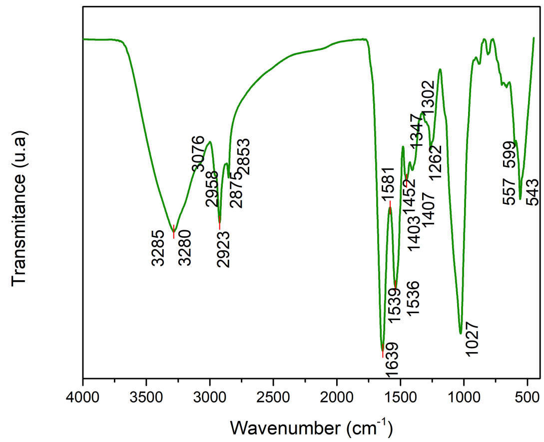

Figure 2. FTIR spectrum of the microalgae *Tetradesmus dimorphus* grown in FBR.

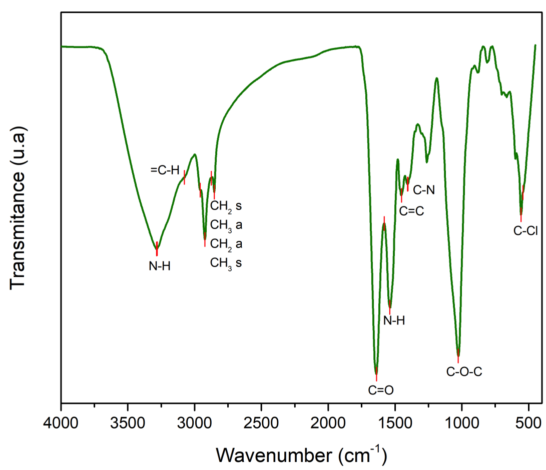

Figure 3. Functional groups identified in the FTIR spectra of the microalgae *Tetradesmus dimorphus* grown in FBR.

metabolism. Similarly, Manzoor *et al.* (Manzoor *et al.*, 2019) reported an increase in biomass productivity from 0.096 g/L day to 0.119 g/L day by adding 5 g of sugarcane bagasse hydrolysate to BBM culture (120 μmol/m^2/s) grown under a 16:8 photoperiod.

In the proposed flat FBR with a settler, 76 ± 0.95 % of the biomass produced by gravitational sedimentation was harvested after 24 h, a lower yield compared to the yields offered by the use of flocculants; among those that have been used for *Chlorella vulgaris* is potassium alum at a concentration of 250 mg/L, with an efficiency of 82.10 % in 5 h, which increased to 92.53 % at pH 5 (Sharma *et al.*, 2016). *Cloelastrun sp* cultures harvested with gravitational sedimentation one night showed a yield of 86.8 ± 7.4 % in a 1.5 L conical FBR and 16 cm in height (May-Cua *et al.*, 2019). The lower yield in gravitational sedimentation obtained in this work is attributed to the height of the FBR; generally, green microalgae have a sedimentation rate of 0.1 m/day (Fazal *et al.*, 2018).

Table 3. FTIR bands found in *Tetradesmus dimorphus* grown in FBR.

Signal	Wavenumber range cm^{-1}	Functional group	Reference
543 547 599	400-800	C-Cl	(Stuart, 2004)
1027	980-1072	C-O-C polysaccharide	(Dilek *et al.*, 2012)
1262 1302	1230-1310	C-N secondary amide	(Smith, 1999)
1347	1191-1356	P=O nucleic acids phosphodiesters	(Dilek *et al.*, 2012)
1403 1407	1390-1430	C-N stretch	(Smith, 1999)
1452	1392-1460	C-O	(Murdock and Wetzel, 2009)
1452	1450-1456	CH_2, CH_3, lipids and proteins	(Dilek *et al.*, 2012)
1536 1539	1515-1570	N-H secondary amide	(Smith, 1999)
1581	1565-1615	C=C, C=N	(Stuart, 2004)
1639	1630-1680	C=O secondary amide	(Smith, 1999)
2853 2875 2923 2958	2850-2960	CH_2 symmetrical, nucleic acids	(Stuart, 2004)
3076	3000-3100	=C-H	(Stuart, 2004)
3280 3285	3170-3370	N-H secondary amide	(Smith, 1999)

Table 4. FTIR spectrum of lipids from *Tetradesmus dimorphus* grown in FBR.

1	2	3	Range	Functional group	Reference
	1039	1041	900-1200	C-O polysaccharide	(Murdock and Wetzel, 2009)
1064	1060	1059			
1403		1404	1392-1460	C-O carboxyl group	
1456	1453	1454	1425-1477	CH_2,CH_3 lipids	(Dilek *et al.,* 2012)
1535	1536	1537	1530-1560	N-H, C-N proteins	(Stuart, 2004)
	1581	1582	1590-1699	COO- carboxyl group	(Murdock and Wetzel, 2009)
1633	1640	1639	1638-1655	C=O amide I	
2852		2852	2850 ± 3	CH_2 symmetric phospholipids	(Stuart, 2004)
2922	2923	2922	2920 ± 3	CH_2 asymmetric phospholipids	
3283	3280	3283	2500-3500	O-H carboxylic acids	(Smith, 1999)

The biomass obtained from the different cultures in the FBR was mixed to obtain a homogeneous mixture and analyzed by FTIR spectroscopy. The signals obtained are shown in Figure 2, and the functional groups identified in the literature are presented in Figure 3. In the FTIR spectra, the term u.a indicates the dimensionless units of transmittance.

Table 3 lists the signals of the analysis, as well as the functional groups to which the biomass of *Tetradesmus dimorphus* cultured in flat FBR corresponds.

The biomass of the microalga *Tetradesmus dimorphus* contains proteins and carbohydrates in addition to lipids due to the nature of the biomass signals, which can be observed at 1027 cm^{-1}, attributed to polysaccharides; these signals were confirmed by the bands at 1581 and 3076 cm^{-1}, nucleic acid bands at 1347, 2853 and 2958, nucleic acid bands, and the presence of proteins, as confirmed by the signals at 1262, 1302, 1403, 1407, 1452, 1536, 1539 1639, 32880, and 3285, and the signal at 1639 cm^{-1} corresponding to the C=O bond in the range of amide I (Murdock and Wetzel, 2009; Stuart, 2004). The overlapping signals of the C=O groups in conjunction with signals at 1581, 3076, and 2853 to 2958 confirm the presence of fatty acids with double bonds. The signals in the range of 543 to 599 cm^{-1} fall within the band corresponding to the C-Cl bond of chloroalkanes, which is attributed to insufficient neutralization of the sodium hypochlorite used in the disinfection step of the FBR.

Grace *et al.* (Grace *et al*., 2020) also reported the presence of signals corresponding to polysaccharides, amides, and even nucleic acids via FTIR spectroscopy of lipids extracted from *Chlamydomonas* sp., *Pseudomueriella* sp. and *Monoraphidium contortum*.

These results are in agreement with those reported for other *Tetradesmus* species. Dileck *et al*. (Dilek *et al.,* 2012) and Zhang *et al*. (Zhang *et al*., 2018) reported the presence of the functional groups C-O-C, P=O, N-H, C=O, CH_2 and CH_3 in the species S. obliquus and S. sp. Arif *et al*. (Arif *et al.*, 2021) also found the abovementioned groups in addition to C-O and C=O in *Tetradesmus dimorphus,* also known as *Scenedesmus dimorphus*.

Lipid extraction was performed in triplicate, the details of the signals obtained are shown in Table 4, and the assignment of functional groups by FTIR spectrometry of *Tetradesmus dimorphus* oil is shown in Figure 4.

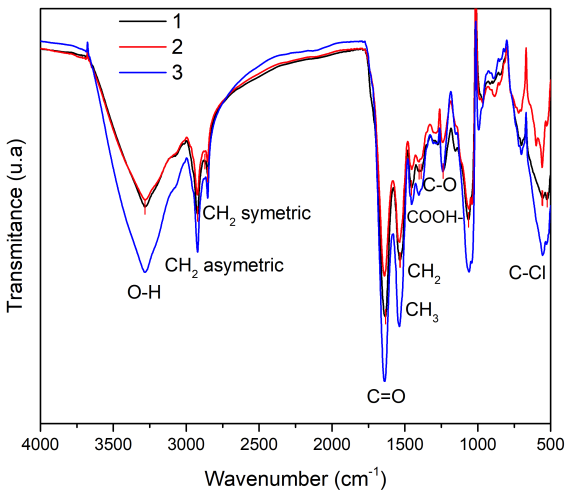

Figure 4. Functional groups identified by FTIR of oil from the microalgae *Tetradesmus dimorphus* cultured in FBR.

For the identification of aliphatic esters, the signals corresponding to the C=O and C-O functional groups, corresponding to the ranges 1730-1750 cm^{-1} and 1100-1300 $cm^{-1,}$ respectively, must be presented (Stuart, 2004). In addition to the three bands, the first band corresponds to the C=O bond between 1735-1750 cm^{-1}, the second band corresponds to the C-C and C-O bonds called C-C-O between 1160-1210 cm^{-1}, which can occur near 1240 cm^{-1} when the extreme carbon is a methyl radical and forms an acetate, and the third band corresponds to the vibration of the O-C-C bond between 1030-1100 cm^{-1}. Therefore, the lipids obtained from the biomass cultivated in the flat-layer FBR for 15 days did not contain esters.

Figure 4 shows the functional groups found via FTIR spectroscopy. The presence of C-O, COO-, and O-H groups of carboxyl groups and CH_2 and CH_3 radicals indicate the presence of carboxylic acids. This technique has not been used for the identification of microalgal lipids; however, the content of carboxylic acids in *Scenedesmus dimorphus* lipids, including palmitic acid, oleic acid, and linolenic acid, has been reported (Kudahettige *et al.*, 2018; C. Zhang *et al.*, 2021).

The *Tetradesmus dimorphus* biomass has potential use in bioethanol production (Chng *et al.*, 2017). On the other hand, it contains the pigments cis-β carotene, trans-β carotene, violaxanthin and lutein (Manzoor *et al.*, 2019). It can accumulate up to 60 % lipids depending on its culture conditions (Marinho *et al.*, 2022). A high lipid content, specifically a high oleic acid content, is essential for biodiesel production (T. Sharma and Chauhan, 2016), and it also stores linolenic and linoleic fatty acids, which are essential fatty acids of importance in animal and human food (Kumar *et al.*, 2021).

Conclusion

It was concluded that the flat FBR with a settler allowed the growth and gravitational settling of *Tetradesmus dimorphus*, culture medium feeding and inoculation in a sterile environment, culture aeration, temperature monitoring and sampling, without the risk of contamination.

This FBR design demonstrated a biomass productivity of 0.016 ± 0.002 g/Ld without the need for illumination or artificial culture media, making it an alternative for reducing the cost of biomass production. It should be noted that the proposed design allows harvesting by gravity sedimentation with 76 ± 0.95 % efficiency, thus eliminating the need for flocculants.

Based on its chemical composition, the biomass obtained could be used in food supplements due to the presence of proteins and carboxylic acids; however, the absence of esters prevents its use in the manufacture of biodiesel.

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