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The cultivation of five microalgae species and their potential for biodiesel production

Mónica Cristina Rodríguez-Palacio^{1,2*} , René Bernardo Elías Cabrera-Cruz¹, Julio Cesar Rolón-Aguilar¹, Ricardo Tobías-Jaramillo¹, Marisol Martínez-Hernández² and Cruz Lozano-Ramírez²

Abstract

Background: Due to the problems we face today, such as wastewater pollution of aquifers and climate change, it is necessary to search for environmental solutions that help us minimize this problem. An alternative solution might be the cultivation of microalgae that are efficient in the purification of wastewater, removal of greenhouse gases and production of biomass that can be used for the production of biofuels such as biodiesel, methane, bioethanol, among others. The aim of this work is to cultivate five strains of microalgae native in Mexico: *Chlorella miniata*, *Coelastrella* sp., *Desmodesmus quadricauda*, *Neochloris oleoabundans* and *Verrucodesmus verrucosus*. The cultivations were performed using municipal wastewater and a foliar fertilizer with the further purpose of assessing their capacity to produce various types of biomass, in particular lipids.

Methods: The experiments were carried out using triplicate 16-L glass bioreactors assays with a 12:12 light–darkness cycle at 25 °C ± 1 under constant aeration. Every 3rd day, a 1-mL sample was taken to determine cell density. In the stationary growth phase, each culture was harvested by sedimentation and lipid content analysis was performed. The biomass with the highest concentration of total lipids was subjected to an analysis of the methyl esters of fatty acids.

Results: An ANOVA test showed significant differences between the growth rates ($F = 6.8$, $p = 0.0001$). The species that were able to produce biomass with the highest concentrations of total lipids were *Coelastrella* sp. with 44–46%; *Verrucodesmus verrucosus* with 43–44% and *Neochloris oleoabundans* 35–37%. As the analysis of the methyl esters of fatty acids showed, the species *Coelastrella* sp. and *V. verrucosus* produced lipids composed of 82.9% and 91.28% of fatty acids, respectively, containing C16–C18 carbon chains.

Conclusions: All the species used in the present study were able to grow on wastewater and produce high concentrations of lipids. Therefore, the demands for biodiesel production could be met in the immediate future after continuing working with different microalgae species. Therefore, it is necessary to determine their adaptation potential to grow on contaminated effluents and produce lipids that can be used for the benefit of people and environment.

Keywords: Phycoremediation, Wastewater treatment with microalgae, Biodiesel from microalgae, *Verrucodesmus verrucosus*, *Coelastrella* sp.

Background

The accumulation of organic pollutants in aquatic ecosystems causes adverse effects on human health and environment. These pollutants are introduced as a result of human activities involving industrial, domestic, livestock and agricultural discharges. As pollution problems increase, research with microorganisms is progressing in order to degrade or neutralize organic pollutants in

*Correspondence: mony@xanum.uam.mx

¹ Division of Graduated Studies and Research, Faculty of Engineering “Arturo Narro Siller”, Autonomous University of Tamaulipas, Ciudad Victoria, Tamaulipas, Mexico

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aquatic systems [1, 2]. Bioremediation is the process by which pollutants are removed from the environment by using organisms or parts of them, and as a result, the pollutants are removed or reduced or as in our case, degraded to simple compounds. This new technology is much cheaper and more environmentally friendly than other ones and is very promising for large-scale problem solving [1, 3].

The principle of cultivation of microalgae used in this study could be compared to that of a biorefinery because it allows for producing many products with a high added value. Industrial production is however, limited by the costs of mineral salts and other nutrients needed for cultivation. Therefore, it is important to keep in mind that wastewater can be a potential culture medium for cultivating these microorganisms due to many “collateral” benefits such as biological treatment of polluted water, production of biomass and biofuels [2, 3].

In 2014, the International Energy Agency reported that 63.7% of the oil produced is consumed by the transport sector, which is equivalent to 22% of the global CO₂ emissions [4]. Therefore, research and development of biofuels has regained importance and those currently under development are biodiesel, produced by the reaction of vegetable oils or animal fats with alcohol; bioethanol produced by the fermentation of organic matter with high starch content and biogas composed mainly of methane, formed by the degradation of organic matter [5, 6].

The biofuels produced are classified according to the raw materials used into first, second and third-generation fuels. The first-generation biofuels are obtained from natural resources from agriculture and food generation, for example, palm, corn, cane, soy, wheat, sunflower. The second-generation biofuels are those produced, for example, from grass, wood, lignocellulosic waste, food waste, and inedible plant crops such as *Ricinus communis* and *Jatropha curcas*. These biofuels might cause problems of deforestation, introduction of invasive species and pests and might threaten biodiversity, compete on land that can be used for food production and are therefore not suitable to fulfill the demand for sustainability [4, 7, 8].

Microalgae and cyanobacteria are considered third-generation biofuel producers. In relation to photosynthesis, they are highly efficient and excellent biomass producers. Moreover, they can be employed in non-fertile soils, or can use wastewater or brackish water for producing high amounts of lipids. Likewise, they are very good CO₂ fixers [4, 8].

A great deal of effort has been made in microalgae biotechnology regarding the treatment of wastewater. This is due to the capacity of adaptation of these microorganisms to this type of effluents, seen in their rapid growth

and capacity to remove nutrients. More than 50 years ago Caldwell [9] and later Oswald et al. [10] suggested the use of massive microalgae cultures for wastewater treatment and protein production simultaneously. Recently, however, this has become more relevant, due to problems of pollution, climate change, population growth, increasing discharges of untreated wastewater and decreasing numbers of clean or pristine water bodies.

Microalgae can be used for tertiary wastewater treatment to achieve better effluent quality through a low energy cost mechanism, where nutrients that were being discharged are nowadays be utilized and metabolized to biomass of high commercial value. According to its chemical composition, this biomass can become a source of fertilizers as well as energy in the first instance [3, 11–13].

Lipids, the raw material for the production of biofuels, are one of the main components of microalgae biomass and their concentrations fluctuate between 20 and 50% of the dry weight depending on each species. The concentration of lipids is also dependent on light intensity, temperature, carbon dioxide concentration and nitrogen presence. However, an important factor for the overall success of biofuel production is the selection of the microalgae strain that should perform optimally in the production of algal biomass and be able to accumulate triacylglycerides and adapt to extreme environments, among others [2, 4, 14, 15]. For this reason, we decided to work with five species of freshwater green microalgae (among them were also those, not have been used in phytoremediation processes until now) and to cultivate them in raw municipal wastewater for testing their adaptation to a complex environment and studying the potential of their biomass for biodiesel production.

Methods

The microalgae chosen in the present work were: *Chlorella miniata*, *Coelastrella* sp., *Desmodesmus quadricauda*, *Neochloris oleoabundans*, *Verrucodesmus verrucosus*. These strains belong to the microalgae culture collection of the Applied Phycology Laboratory at UAM Iztapalapa, México (Fig. 1).

These microalgae were selected due to previous studies where they showed rapid growth and accumulation of lipids, when cultivated on conventional culture media F/2 [11, 16] and in the foliar fertilizer Bayfolan forte [3].

All microalgae were evaluated in batches in triplicate where a 20% (v/v) inoculum was taken from exponential phase cultures. The growth of each microalga was maintained in 16-L glass photobioreactors with aeration through injection pumps in a range of 0.6 vvm (volume of air per total volume of the bioreactor per minute) and at an irradiance of 50 mmol/m²/s. The experiments were

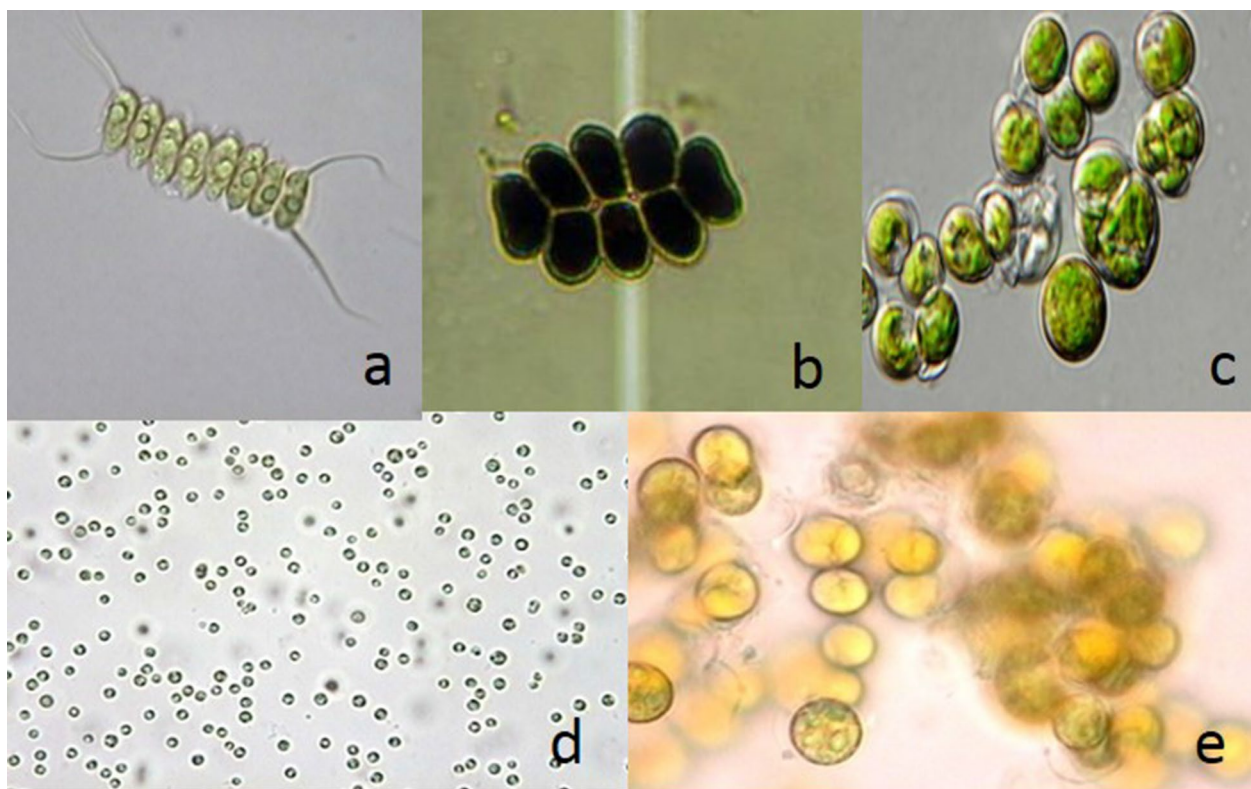


Fig. 1 Optical microphotographies of the species used in the present study: **a** *Desmodesmus quadricauda*, **b** *Verrucodesmus verrucosus*, **c** *Neochloris oleabundans*, **d** *Chlorella miniata*, **e** *Coelastrella* sp

Table 1 Bayfolan forte culture medium (BF)

Compound	% (p/v)	Compound	% (p/v)
Total nitrogen (N)	11.470	Phosphorous oxide (P_2O_5)	8.000
Potassium oxide (K_2O)	6.000	Boron (B)	0.036
Copper (Cu)	0.040	Iron (Fe)	0.050
Molybdenum (Mo)	0.005	Zinc (Zn)	0.080
Thiamine hydrochloride	0.004	Sulfur (S)	0.230
Calcium oxide (CaO)	0.025	Cobalt (Co)	0.002

One milliliter of Bayfolan Forte was added to 1 L of distilled water

carried out for 30–60 days and the algal biomass was subsequently harvested. For harvesting, aeration of the reactors was suspended and the cultures were placed in a refrigerator at 4 °C to accelerate algal sedimentation.

The growth of each microalgae strain was monitored every third day by using a Neubauer camera with an optical microscope [16].

The following culture media were used: leaf fertilizer Bayfolan Forte © (Table 1) and municipal wastewater.

The samples of treated municipal water were collected from an anaerobic UASB reactor (Experimental Pilot

Table 2 Composition of the culture medium

Culture medium	Nutrient concentrations	
	N-[NH_4]	P-[PO_4^{-3}]
Bayfolan forte	11.47	8.0
Wastewater	22.0	8.0

Plant No. 9 of the UAM-I), where the levels of ammonium concentration ranged from 20.5 to 40.8 mg/L and the orthophosphates ranged from 4.9 to 16.5 mg/L [3]. The samples were irradiated with UV light for a period of 48 h, and for all the experiments this effluent treated at 100% (v/v) was used. The final composition of this culture medium is shown in Table 2.

The percentage of lipids was determined by the method proposed by Bligh and Dyer [17]. The results obtained were subjected to an analysis of variance (ANOVA) in order to evaluate the differences in the treatment. The statistical program SYSTAT 9 was used. The resulting total lipids and the profiles of the methyl esters of fatty acids (FAMES) were analyzed in a gas chromatograph with a flame ionization detector (FID) (HP6890) [18].

Table 3 Maximum cell growth and harvested biomass in the different culture media

Days	Microalgae	Cells/mL		gr/L	
		WW	BF	WW	BF
21	<i>V. verrucosus</i>	93×10^6	95×10^6	4	4.3
26	<i>Coelastrella</i> sp.	50×10^6	52×10^6	3	3.2
53	<i>N. oleoabundans</i>	45×10^5	47×10^5	2.8	2.9
59	<i>C. miniata</i>	20×10^5	25×10^5	1	1.1
59	<i>D. quadricauda</i>	59×10^5	66×10^5	3.2	3.5

Table 4 Lipid content of the biomass cultivated in WW and BF

Microalgae species	WW	BF
<i>Chlorella miniata</i>	27.3	25.5
<i>Coelastrella</i> sp.	46.9	44.6
<i>Desmodesmus quadricauda</i>	26.5	25.3
<i>Neochloris oleoabundans</i>	37.3	35.5
<i>Verrucodesmus verrucosus</i>	44.6	43.5

Results and discussion

The cultures did not reach maximum cell growth at the same time, after 21 days, *Verrucodesmus verrucosus* reached maximum growth, followed at 25 days by *Coelastrella* sp. (Table 3).

Using the cell growth data for every species, a non-parametric Kruskal–Wallis ANOVA test was performed resulting in a value of $p = 0.003$ $\lambda = 0.05$, and the analysis of multiple comparisons showed significant differences between *Coelastrella* sp. and *Verrucodesmus verrucosus* compared to all other species.

According to the statistical analysis of the variance (ANOVA) the results regarding the lipid content produced by the strains cultivated in foliar fertilizer (BF) and on municipal wastewater (WW) (Table 4) were not found significantly different ($p = \leq 0.05$).

The species generated the highest concentration of total lipids were *Coelastrella* sp. with 44–46%; *V. verrucosus* with 43–44% and *N. oleoabundans* 35–37%. The *Neochloris* species were extensively studied for their ability to accumulate lipids in conventional culture media. Lipid concentrations between 35 and 54% and 46.1 and 57.7% were reported for these species [7]. It should however be noted that these concentrations were achieved using ideal culture media, and not on municipal wastewater. Recently, it has been reported about the potential of *V. verrucosus*, not only to store lipids, but also to bioremediate pollutant effluents such as organic leachate and wastewater [19–21] *Coelastrella* sp. is a species that has a high potential to accumulate lipids and other bioproducts

such as betacarotenes [22, 23]. The percentages of lipids produced reached 46.9 and 44.6%, and were higher than those reported by Luo et al. [24], who achieved values of 22.4% and 25.5%; and Dimitrova et al. [22], who reported a percentage of 37% [22].

For the species which produced the highest percentages of total lipids, we performed an analysis of fatty acids to determine their potential for biodiesel production. As we know, the main components of the lipid fraction are triacylglycerols, free fatty acids, waxes, sterols, hydrocarbons, glycolipids (predominant in chloroplastic membranes), phospholipids (abundant in plasmalemma and diverse endomembranous systems) and pigments (carotenoids, chlorophylls, phycobilins, etc.) [16, 25–27]. Not all microalgal lipids are suitable for biodiesel production, however, the appropriate ones (fatty acids, free and covalently bound to glycerol and its derivatives) are frequently produced and constitute the largest fraction of total lipids, usually from 20 to 40% [7, 25].

According to the results, the total lipids of *Verrucodesmus verrucosus* are composed of 91.28% of fatty acids with C16–C18 carbon chains that are highly recommendable for the production of biodiesel [28], whereas the percentage of linoleic acid (C18:3) is below 12%, which meets the quality standards for fatty acids precursors of biodiesel (Fig. 2) [29].

When *Coelastrella* sp. is used, the total lipids are composed of 82.9% fatty acids with C16–C18 carbon chains, the percentage of C18:3 is less than 12% which makes the biomass of this microalgae species also suitable for biodiesel synthesis (Fig. 2).

Figure 2 depicts the result of *Neochloris oleoabundans*, where an increase in fatty acids with unsaturated chains (C18:2) is observed and, in the case of C18:3 its percentage exceeds the limit of 12% which is a disadvantage in terms of the quality criteria established by law, although there was no presence of polyunsaturated fatty acids (presence of 4 or more double bonds in the chains). The high percentage of C18:3 makes the biomass unsuitable for producing biodiesel (Fig. 2). It is worth mentioning that the presence of double bonds in fatty acid chains affects the quality of biodiesel, making its chemical structure more reactive to free radicals [29].

Conclusions

All species used in the present study were able to grow on wastewater and produce high concentrations of lipids, above 25%. Among them the *Coelastrella* sp., *Verrucodesmus verrucosus* and *Neochloris oleoabundans* species showed better results than the others. Therefore, the first two species are good candidates for the production of biodiesel due to their fatty acids type produced and the amount of biomass obtained in less time.

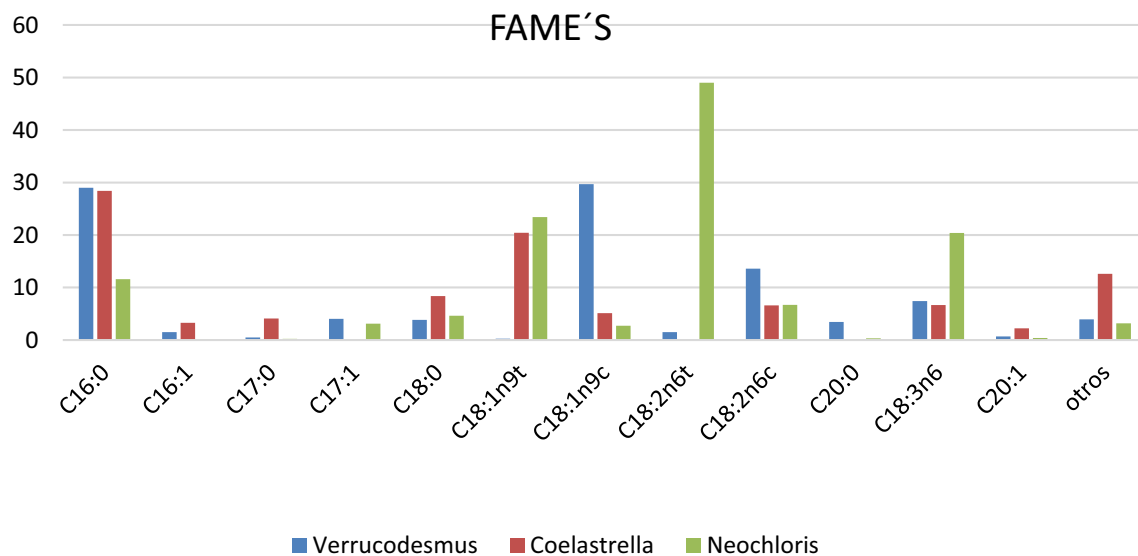


Fig. 2 Comparison of the FAMES profile of the three species

It is necessary to continue working on a laboratory and pilot scale with native and little studied species, such as those used in the present study, in order to determine their potential of adaptation to growth on contaminated effluents and to evaluate the total lipid production. These sustainable energetic and ecological alternatives should be implemented during a large-scale cultivation in the near future to achieve virtuous production circles, such as cleaning a polluted effluent, capturing greenhouse gases and producing biomass that can be used for the production of biodiesel or other value-added bioproducts.

Abbreviations

BF: Bayfolan forte media; WW: Wastewater; FAMES: Fatty acid methyl esters; ANOVA: Analysis of variance.

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Authors' contributions

All sections of the study, including concepts, experimental design and laboratory work, were conducted with the participation of the authors. All authors contributed to the conclusions, the summary and the perspective of the study. All authors read and approved the final manuscript.

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Availability of data and materials

Data and additional materials are available on request.

Declarations

Ethics approval and consent to participate

The authors declare that they complied with the ethical guidelines.

Consent for publication

The authors declare that they give consent for publication.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Division of Graduated Studies and Research, Faculty of Engineering "Arturo Narro Siller", Autonomous University of Tamaulipas, Ciudad Victoria, Tamaulipas, Mexico. ²Applied Phycology Laboratory, Hydrobiology Department, Metropolitan Autonomous University, P.O.B. 55-535, 09340 Mexico City, Mexico.

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