



ISSN: 2230-9926

Available online at <http://www.journalijdr.com>

# IJDR

International Journal of Development Research

Vol. 11, Issue, 07, pp. 48393-48398, July, 2021

<https://doi.org/10.37118/ijdr.22330.07.2021>



RESEARCH ARTICLE

OPEN ACCESS

## PRODUCTION OF SCENEDESMUS SP. MICROALGAE IN CASSAVA (CASSAVA WASTEWATER) FOR EXTRACTION OF LIPIDS

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### ARTICLE INFO

#### Article History:

Received 27<sup>th</sup> April, 2021

Received in revised form

09<sup>th</sup> May, 2021

Accepted 06<sup>th</sup> June, 2021

Published online 25<sup>th</sup> July, 2021

#### Key Words:

Microalgae; *Scenedesmus sp.*;  
Cassava; Lipid Extraction, Lipid profile.

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### ABSTRACT

Microalgae are a renewable energy source and have been studied to obtain several bioproducts among which are lipids that can be applied in energy and food sectors. The production of lipids from microalgae has some bottlenecks such as their nutritional requirements, cultivation mode, and the need to break down the cell structure since lipid is an intracellular product. In light of this problem, this research seeks to analyze factors that influence the growth processes of *Scenedesmus sp.* microalgae biomass in the synthetic ASM-1 medium supplemented with cassava, an effluent from the pressing of cassava, and thus to assess the cultivation conditions, dry biomass, and lipid content through the study of cell lysis and forms of extraction. *Scenedesmus sp.* was cultivated in different concentrations of the effluent. Two types of pre-treatments for recovery of lipid (ultrasound and autoclave), two solvent systems (chloroform/ethanol and chloroform/methanol), and two methods of extraction (Bligh and Dyer with ultrasound and Soxhlet) were evaluated for cell disruption. *Scenedesmus sp.* microalgae showed better efficiency in dry biomass production in culture mediums supplemented with cassava (5% - 10%) compared to the synthetic growth medium. It was observed that for both solvent systems autoclave pre-treatment was more efficient; however, the Bligh and Dyer method adapted with the use of ultrasound and chloroform/ethanol solvent systems obtained the higher yield of 19.27% and lower cost of thermal energy, when compared to Soxhlet with the same solvent system that resulted in 15.71% of total extracted lipids. Analysis of the lipid profile suggests the possibility of using microalgae oil for biodiesel production due to low unsaturated content, lower than that of vegetable oils (soybean, cotton, and cinnamon).

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Citation: Keilla S. Cerqueira, Diego F. Coêlho, Jacqueline R.S. Rodrigues and Roberto R. Souza. "Production of scenedesmus sp. microalgae in cassava (cassava wastewater) for extraction of lipids", *International Journal of Development Research*, 11, (07), 48393-48398.

## INTRODUCTION

Microalgae are characterized as photosynthetic microorganisms that combine water, carbon dioxide, and sunlight to produce various forms of energy (Bresolin, 2018). The unicellular structure of microalgae allows them to easily convert solar energy into chemical energy; they capture CO<sub>2</sub> from air and use it as a source of carbon in their metabolism. According to Bhalamurugan, Valerie (Bhalamurugan, 2018), this biochemical conversion is being used commercially to obtain microalgae biomass and, consequently, products with commercial application. For microalgae biomass to be more economically viable compared to traditional cultures as a raw material for biofuel production it is necessary to increase biomass productivity and lipid content and reduce the costs of inputs used in the preparation of the growth medium. For this purpose, the production of

microalgae biomass can also be achieved in alternative culture mediums, among which it is possible to highlight cassavaan effluent from the pressing of cassava (Neves, 2016). Effluents are generated during the processing stages of cassava, resulting from root cleaning water and water from pressing of cassava with the latter commonly referred to as cassava. Nutrients that are essential for microalgal growth such as sugars (sucrose, glucose, fructose, and maltose), nitrogen, potassium, mineral salts, glycosides are present in this effluent. The production of biofuels from microalgae using cassava effluent is primarily based on high biomass production. For biodiesel production this biomass also needs to have high percentage of lipids. The accumulation of lipids in microalgae is attributed to consumption of sugars at a higher rate than a cell generation rate, which promotes the conversion of excess sugar into lipids (Richmond, 2017). The aim of using cassava is to minimize costs of a synthetic medium and to

utilize already available nutrients of the potentially contaminant effluent. *Scenedesmus sp.* is microalgae of rapid growth and high capacity to adapt to conditions of cultivation, using several sources of nitrogen and growing in a temperature range between 15 and 40°C (Chew, 2018). The ease of cultivation and handling drives the industrial application of this type of microalgae in both food and fuel sectors. They can accumulate substantial amounts of lipids (approximately 20-50% of dry weight) (Omirou, 2018). The lipid content of microalgae *Scenedesmus sp.* ranges from 11 to 20% when grown under standard conditions and can reach 70% when the growth medium is optimized (Fernandes, 2017). Since lipids bioaccumulate intracellularly, their extraction requires a process known as cell disruption or cell lysis. Cell disruption is the first step in the separation of intracellular products (Aguzzi, 2016). It involves the release of intracellular compounds through the application of several methods such as ultrasound, abrasive treatments such as grinding, chemical treatment, freezing, osmotic pressure variation, microwaves, and autoclaving, among others. Even though there are several specific methods of chemical and enzymatic disruption, it is the mechanical methods that have found application in the pilot and industrial scales (Gröger, 2016). The efficiency of a disruption method is generally evaluated by its degree of cell disruption and the time in which the disruption is achieved (Leitão, 2017).

The cell lysis process is fundamental in optimizing lipid recovery during the extraction process, which is started soon after the cell disruption. In lipid extraction from microalgae various processes are used to increase efficiency and yield at low cost. Lipids can be extracted by chemical processes in which solvents such as benzene, ether, or hexane are used to promote the lipid extraction. The use of enzymes is another effective extraction method that makes oil fractionation easier (Franco *et al.*, 2013). According to Aenehvand, Toudehrousta (Aenehvand, 2016), the most efficient and applied method is solvent extraction, with the determining factor being the type of solvent system to be used. The lipids obtained from microalgal biomass have mixed composition of fatty acids – according to its polarity – therefore it is recommended to use a system consisting of more than one solvent, polar and non-polar (de Jesus, 2018). In general, among the main factors that influence the lipid extraction are the efficiency of cell lysis, total volume and the proportion of polar/non-polar solvents contained in the system, and the type of contact between fluid and biomass. Microalgae have been considered one of the most promising raw materials for biofuel that can meet the challenges of energy security and environmental protection (Wang *et al.*, 2018).

To reduce the cost of production, increase or equate the productivity of biomass and lipids to that of a synthetic ASM-1 medium, a microalgae cultivation in *cassava* was developed as a supplement to the synthetic medium. To achieve this goal, the work was divided into different stages: standardizing the growth of microalgae *Scenedesmus sp.* in the synthetic ASM-1 medium to determine concentrations of nitrogen, phosphorus, light exposure time, period of cultivation and biomass production; cultivating microalgae in the alternative culture medium formulated from *cassava* dilutions in ASM-1; establishing the best lipid extraction technique, combining pre-treatment and solvent systems, using dry biomass; comparing biomass productivity and total lipids of the cultures in the ASM-1 medium with the alternative medium; analyzing the lipid profile of microalgae *Scenedesmus sp.* grown in the ASM-1 medium and in additional mediums supplemented with *cassava*.

## MATERIALS AND METHODS

**Microalgae:** The microalgae species used in this study, *Scenedesmus sp.*, was obtained from a sample supplied by the bank of strains from the Laboratory of Environmental Biotechnology (LABAM) of the Federal University of Sergipe (UFS). The mediums were prepared with analytical reagents of 98% purity and distilled water, stored in amber bottles, and kept at ambient temperature 25°C.

**Growth medium:** At the laboratory, the microalgae were grown in the ASM-1 medium at standard concentrations of the medium (15) and in *cassava*. The *cassava* used in this research to supplement the synthetic growth medium was collected in the flour houses of the region of Riachão do Dantas, Brazilian municipality of the state of Sergipe (latitude 11°04' 08" South and longitude 37°43' 30" West). The collected effluent was transported in a 20-liter plastic container, then stored in 2-liter plastic bottles, all sterilized, and kept in a refrigerator (-10°C) to preserve its physico-chemical characteristics. Table 1 shows the composition of *cassava* residue used as a supplement to the synthetic culture medium.

**Table 1. Composition of cassava used in this work**

Components	Concentration
Starch (%)	6.1
Carbohydrates (%)	3.64
Reducing sugars (g/100mL)	2.04
Non-reducing sugars (g/100mL)	1.53
Invert sugar (g/100L)	2.04
Total nitrogen (mg/L)	2240
Total phosphorus (mg/L)	461
Total sulfur (mg/L)	0.010
Total zinc (mg/L)	0.081
Total manganese (mg/L)	0.500
Total iron (mg/L)	0.701
Total copper (mg/L)	0.021
Total calcium (mg/L)	25.63
Magnesium (mg/L)	33.20
Potassium (mg/L)	11.02
Total cyanide (mg/L)	48.6
pH	6.4

The mediums prepared with the effluent were autoclaved at 120°C and 1 atm pressure, passed through filter paper by simple filtration and diluted in various concentrations to obtain the concentrations presented in Table 2. Autoclaving was required to sterilize the medium, reduce the organic load, eliminate the cyanide that could still be present in the medium, reduce the effluent turbidity and, thus, prevent illumination dispersion in the medium, and allow to verify in which concentration the microalgae would develop similarly or superior to that of the ASM-1 medium.

**Table 2. Different concentrations of cassava diluted in ASM-1 medium for cultivating *Scenedesmus sp.* using Erlenmeyer photobioreactors**

Treatment	Effluent %
1*	0*
2	5
3	10
4	20
5	40
6	60
7	80
8	100

\*control cultivation with ASM-1 medium without addition of effluent

**Determination of biomass and preparation of inoculums:** Microalgae cultivation was performed on a laboratory scale in 500 mL Erlenmeyer flask with the initial volume of previously autoclaved ASM-1 medium of 400 mL under constant luminous flux of 30  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , constant forced aeration of 2  $\text{L}\cdot\text{min}^{-1}$ , and temperature of 26  $\pm$  4°C. The biomass concentration ( $\text{g}\cdot\text{L}^{-1}$ ) had been determined daily using a spectrophotometer to read the absorbance at a wavelength of 750 nm. Absorbance readings were taken for different cell concentrations, from this data a calibration curve was constructed, and through the linearization equation ( $y=5.7092x$ ) the algae concentration in the experiments was calculated. The samples were kept until they reached a steady state of growth and, subsequently, centrifuged at 3000 rpm for 10 min to collect the biomass. The biomass was dried in an oven for 24 hours, macerated, and stored for further extraction.

To determine the quantity (mL) of the microalgae suspension to be used as inoculum, the biomass was quantified in g.L<sup>-1</sup>, then the volume needed to inoculate 400 mL of cultivation with a biomass started at approximately  $\pm 0.1$  g.L<sup>-1</sup>.

**Extraction of lipids:** The following methodology aims to extract the lipids contained in the biomass of microalgae *Scenedesmus sp.* Among the employed methods are the method proposed by Bligh and Dyer (16), modified using an ultrasound, and the Soxhlet method proposed by Ramluckan, Moodley (17). The chosen solvent system, as well as its proportions, is based on studies of Ramluckan, Moodley (17) that demonstrate the properties of the thirteen solvents used for the extraction of lipids from the algal biomass. The homogenization method proposed by Bligh and Dyer (16) was optimized by Zorn, Pedro (18) through the use of ultrasound and this adaptation was inserted in the following experiments. The moisture content of the biomass applied in the experimental procedure was selected based on the best extraction results observed in the studies of Zorn, Pedro (18). As a proposal for improvement in the sample pre-treatment using an autoclave, the pre-treatment time was longer than the methodology used by Lee, Yoo (19), shown in Table 3.

**Table 3. Factors used based on the best results of Lee, Yoo (19) and Zorn, Pedro (18)**

Factors	
Moisture	64%
Total volume of solvents	33 mL
Solvent ratio	1:1
Ultrasound time	30 min
Autoclave time	15 min

In order to control the quantity and hydration of the sample, 1 g of the dry biomass was weighed in a 125 mL Erlenmeyer flask. The mass was hydrated to 64% of moisture and the solvents were in 1:1 ratio totaling 33 mL. The parameters and methods used for extraction are detailed in Table 4.

**Table 4. Parameters and methods to be used for extraction**

Pre-treatments	30 min ultrasound 15 min autoclave
Solvent systems	CM - chloroform/methanol (1:1) CE - chloroform/ethanol (1:1)
Extraction methods	Bligh and Dyer (16) with ultrasound Soxhlet

Two types of pre-treatment were used to breakdown the cell structure: ultrasound, the hydrated sample was placed in the bath with ultrasound for 30 minutes at room temperature; and autoclave, the Erlenmeyer flask containing the hydrated sample was placed in the autoclave under 1 atm pressure and temperature of 121°C for 15 minutes. In the adapted Bligh and Dyer (1959) extraction method, chloroform and methanol (or ethanol) were added to the hydrated sample in the proportions and total volumes pre-established in Table 4. The system was mixed manually for 2 minutes and then placed in the ultrasound bath for 25 minutes at room temperature; the Erlenmeyer flask was removed from the ultrasound and the mixing and ultrasound procedure was repeated three times. In Soxhlet extraction, according to the experimental planning, the hydrated sample was added to the 100 mL flask of the solvent system in the previously established 1:1 ratio. The mixture was placed in the Soxhlet system. The extraction time was 3 hours, according to the studies of Ramluckan, Moodley (2014). After the extraction, the content was filtered in Erlenmeyer flask through filter paper with an average diameter of 12.5 cm, then the filtrate was collected, transferred to a 125 mL separation funnel and left to rest until the complete separation of the phases. After the separation, the lower phase of the funnel, corresponding to the solvent, in both processes was removed and evaporated in the oven at temperature of 55-60°C for 24 hours.

The contents were then dried, cooled to room temperature for 15 minutes, and weighed again to determine the total of extracted lipids.

The following equation was used to calculate the percentage of lipids in 1 g of dry biomass:

$$\% \text{ Lipids} = \frac{(m_2 - m_1)}{\text{weighted biomass (g)}} \cdot 100$$

Where  $m_2$  is the mass of the flask containing the lipids and  $m_1$  being the mass of the empty flask.

### Characterization of lipids

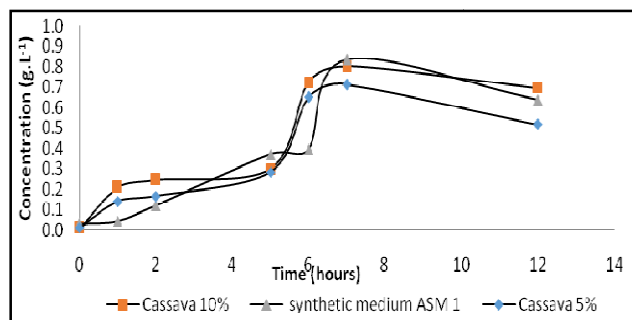
**Derivatization of vegetable oils and fats:** About 50 mg of the sample of microalgae oil was saponified with 4 mL of 2% NaOH methanolic solution under constant mixing in a magnetic stirrer and heating for 5 minutes. 6 mL of esterifying solution prepared from 8.29 g of ammonium chloride (NH<sub>4</sub>Cl), 250 mL of methanol, and 13.6 mL of concentrated sulfuric acid was added under agitation and heating for another 3 minutes. At the end of that time 5 mL of impregnated sodium bicarbonate was added. Finally, after 2 more minutes, 5 mL of hexane was introduced, and the mixture was stirred and heated for further 2 minutes. At the end of the process, the phase of the solvent containing the monoester was collected with a Pasteur pipette, and had enough hexane added to fill a 100 mL volumetric flask. The mixture was then stored for analysis by quantitative gas chromatography while the aqueous phase was discarded.

**Chromatographic analysis:** The chromatographic profile of fatty acids was performed by CEPPA. The gas chromatography analyses were performed on a VARIAN chromatograph model CP3900 with *split/splitless* injector with flame ionization detector (FID). The column used was CpSil for FAME filled with polyethylene glycol – 100 m (length) x 0.25 mm (internal diameter) x 0.39  $\mu$ m (film thickness). The stripping gas was N<sub>2</sub> maintained at pressure of 40 psi. The injection volume was 1.0  $\mu$ L. The oven was programmed to work in the isothermal mode at 210°C and the detector temperature was 260°C with the total running time being 70 minutes. A Sigma Supelco FAME standard containing 37 components and at 10 mg. mL<sup>-1</sup> was used for the detection of fatty acid profiles in the samples. Starwork Station 5.0 software was used for data collection. The retention times of fatty acids in the sample were compared with the standard retention times, standardized and expressed in g. 100g<sup>-1</sup>.

## RESULTS AND DISCUSSION

**ASM-1 cultivation supplemented with cassava:** The *Scenedesmus sp.* microalgae were cultivated for a period of 12 days. During that time daily growth of the dry biomass was monitored, according to the procedure described in section 2.3, in order to evaluate the growth and estimate the ideal period for the next cultures. In different concentrations of the ASM-1 medium supplemented with cassava above 10% the microalgae did not show growth over the time of cultivation. In the experiments with the higher concentration of cassava the microalgae growth was inhibited while the most effective growth occurred in the dilutions of 5% and 10%.

**Biomass production:** Since the cultivation in the alternative medium prepared with 10% cassava showed similar behavior when compared to the synthetic ASM-1 medium, more than one group of experiments with 10% and 5% of the effluent was carried out for confirmation. Figure 1 illustrates the biomass growth in the synthetic ASM-1 medium and in the effluent of 10% and 5% during the 12 days of cultivation. Regarding productivity, although the cultivation with the effluent showed higher values of biomass relative to ASM-1 in the peak phase of the experiment, it did not show a significant difference at the end of the cultivation.



**Figure 1. Growth of microalgae in concentration of dry biomass in ASM-1 medium and cassava with 5% and 10% dilution**

## Extraction of lipids

**Comparison of pre-treatment methods for extracting microalgae lipids:** The factors that influence the process of cell lysis and microalgae extraction were examined by comparing two extraction methods (adapted Bligh and Dyer and Soxhlet), two solvent systems (CE–chloroform/ethanol and CM–chloroform/methanol), and two types of pre-treatments (ultrasound and autoclave) to perform the molecule breakdown. The experimental procedure for obtaining lipids was carried out in two steps. The data from the first step, corresponding to the extraction based on the method of Bligh and Dyer (Bligh, 1959) with the use of ultrasound and autoclave, is presented in Table 5.

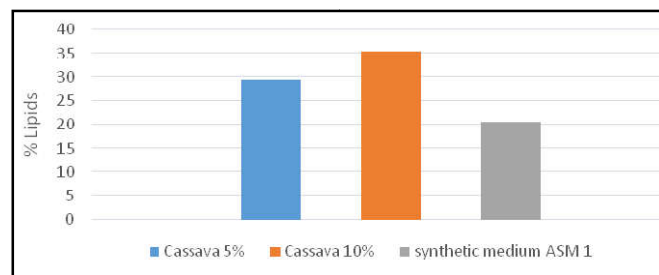
**Table 5. Extraction data based on the Bligh & Dyer method (1959) with the use of ultrasound and autoclave**

Solvent system	Pre-treatment	% of lipid in microalgae
CM*	Ultrasound (30 min)	10.78
(1:1)	Autoclave (15 min)	19.94
CE*	Ultrasound (30 min)	17.31
(1:1)	Autoclave (15 min)	19.27

\*CM-chloroform/methanol; \*CE-chloroform/ethanol

Notably, the best results for both solvent systems were with the cell lysis performed in the autoclave. These results contradict the results obtained by Lee, Yoo (2010) in which fractions of lipids in dry biomass amounted to 5% with autoclave. Between the two types of pre-treatment compared in this experiment, the ultrasound was the best to promote cell disruption. This divergence of results lies in the autoclave time because the methodology by Lee, Yoo (19) uses only 5 minutes and in this experiment 15 minutes were used. According to Andrade (Andrade, 2014), the total fraction of lipid from *Scenedesmus sp.* corresponds to a range of 19.6% to 21.1% by weight of dry biomass, so the studies are within the margin of literature. The largest fraction of lipid extracted from microalgae *Scenedesmus sp.* was obtained with the CE system. The best extraction performance using ethanol is due to the lower polarity of ethanol compared to the polarity of methanol, the fact observed through the lower dielectric constant of ethanol. The lipids are usually weakly polar with dielectric constants in the range 2 to 4 so less polar solvents yield higher solubilization rates of lipids (Navarro López, 2016).

**Production of lipids:** The production of lipids by microalgae was analyzed at the end of the cultivation. For this, a sample of each experiment was collected and the concentration of the lipid was determined by Bligh and Dyer (Bligh, 1959) extraction. The results demonstrated in Figure 2 represent the lipid fractions of independent experiments. It can be observed from the analysis of Figure 2 that the largest fraction of lipids in the microalgae biomass grown with cassava was found in cultures with 24 hours of exposure to light, particularly in the 10% effluent (35.5% of lipids). All the experiments produced a higher percentage of lipids than ASM-1 and this can be explained by the increase of available nutrients from the addition of cassava.



**Figure 2. Percentage of total lipids in microalgae biomass grown in ASM-1 and in cassava.**

When using agroindustrial waste in biotechnological processes it is considered more beneficial to use high concentrations due to the waste biodegradation process that occurs during the system and reduces the environmental impact of the material when released into the environment. However, the pure residue is not always a suitable environment for growth of microorganisms, a fact that has been true with *Scenedesmus sp.* microalgae. Cassava is a liquid effluent of yellowish color and with high turbidity which produces a scattering of light due to the presence of colloidal or suspended particles, the light that is a limiting factor to the growth of microalgae.

**Lipid profile:** The fatty acid composition of the lipid material of the microalgae species *Scenedesmus sp.* grown in the synthetic culture medium (ASM-1) and in 10% and 5% cassava supplemented mediums was analyzed with gas chromatography which identified 16 fatty acids with different degrees of unsaturation and with chain size ranging from C16 to C20. According to Pleissner, Lau (22), fatty acids commonly found in all of microalgae are: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1 cis9), hexadecenoic (C16:1 cis11), hexadecadienoic (C16:2 cis7, cis10), heptadecenoic (C17:1 cis9), stearic (C18:0), oleic acid (C18:1 cis9), vacenic (C18:1 cis11), and linoleic (C18:2 cis9, cis12). The fatty acid profile of *Scenedesmus sp.* is composed mostly of saturated and mono-unsaturated fatty acids, among which are margaric acid (26% to 30%) and oleic acid (31% to 38%), respectively. Regarding the forms of cultivation, the significant difference in the types of fatty acids produced is, essentially, in the saturated fatty acids, in which there was a 10% drop when grown in cassava.

Moreover, a considerable presence of fatty acids with three or more double bonds (19% to 24%) was observed in these samples. The lipid profile directly influences the purpose of the oil. When used for biodiesel production, for example, the unsaturation reduces viscosity and improves cold filter plugging point. However, the presence of unsaturated fatty acids will cause low oxidative stability to biofuel. High-quality biodiesel requires high levels of saturated and monounsaturated fatty acids. The presence of polyunsaturated compounds is not desirable as it decreases oxidative stability, when present in large quantities, due to high reactivity in the presence of oxygen, increasing viscosity and causing corrosion. In general, the acids most synthesized by the species independent of the cultivating conditions were oleic (C18:1) of the monounsaturates, linoleic and linolenic group (C18:2 and C18:3) of the polyunsaturates, and the margaric (C17:0) of the saturates. Table 6 shows the fatty acid composition of the microalgae *Scenedesmus sp.* grown in the synthetic ASM-1 medium and in the cassava supplemented medium compared to three other raw materials (soybean, canola and palm) currently used for biodiesel production. It was observed that the results found for the saturated fatty acids of microalgae *Scenedesmus sp.* are similar to the results found for palm oil which is widely used in cooking. For polyunsaturated fatty acids the microalgae showed lower values than soybean oil, cotton, and canola used for biofuel production. The obtained results suggest a possibility of using microalgae oil for biodiesel production due to the low unsaturated content which is lower than that of vegetable oils (soybean, cotton, cinnamon).

**Table 1. Fatty acid profile of microalgae *Scenedesmus sp.* grown in synthetic ASM-1 medium and in 5% and 10% cassava determined with Varian gas chromatograph with split/splitless injector with flame ionization detector (FID)**

Fatty acids	ASM-1	5% Cassava	10% Cassava	Soy	Cotton	Canola	Palm
C12:0	0	0	0	0	0	0	0.18
C14:0	0	0	0	0.06	1.5	0.06	0.71
C15:1	0	0	0	9	0	0	0
C16:0	0	0	0	9.9	25	3.75	41.86
C16:1	0.74	0.99	1.13	0.08	0	0.21	0.14
C17:0	29.25	26.93	28.37	0.1	0	0.04	0.09
C17:1	0.61	0.42	0.31	0.08	0	0	0
C18:0	0.21	0.52	0.31	3.94	1.72	1.87	4.86
C18:1	39.8	41.78	40.49	21.35	28	62.41	42.09
C18:2	9.03	10.89	10.91	56.02	40	20.12	8.67
C18:3	10.25	13.07	12.38	7.15	0.5	8.37	0.22
C20:0	0.35	0.66	0.54	0.41	0	0.64	0.37
C20:1	0.89	0.7	0.56	0	0	1.54	0.16
C20:2	0.3	0.29	0.27	0	0	0.11	0
C20:3	0.23	0.07	0.1	0	0	0	0
C20:4	0.23	0.21	0.21	0	0	0	0
C22:0	0	0	0	0	0	0.35	0.06
C23:0	11.5	3.47	4.42	0	0	0	0
C24:0	0	0	0	0	0	0.27	0.08
C24:1	0	0	0	0	0	0.26	0
SFAs*	42.38	32.28	34.26	14.41	28.22	6.98	48.21
MUFAs*	36.35	43.76	42.45	21.51	28	64.42	42.39
PUFAs*	19.28	23.96	23.29	63.17	40.5	28.6	8.89
Unsaturated content	1.31	2.10	1.92	5.88	2.43	13.33	1.06
SOURCE	Author	Author	Author	Zambiasi, Przybylski (2007)	Dantas (2006)	Zambiasi, Przybylski (2007)	Corsini, Jorge (2008)

\*SFAs - saturated fatty acids \*MUFAs - monounsaturated fatty acids \*PUFAs - polyunsaturated fatty acids

According to Kumar (Kumar, 2017), for the oil to be of good quality it must have little polyunsaturated fatty acids in its structure as they cause oxidative instability that requires antioxidants which raise the cost of biodiesel.

## CONCLUSIONS

This work evaluated cell density, cultivation conditions, dry biomass, and lipid content of *Scenedesmus sp.* microalgae grown in the synthetic ASM-1 medium, and in the alternative culture medium supplemented with cassava (a residue from the flour houses of the region) in order to define a concentration of the effluent favorable to the microalgae growth in terms of biomass and total lipids. The experiments showed that the combination of pre-treatments performed on microalgae biomass and, thus, the use of solvent systems employed in this work, facilitated the extraction of the lipids from *Scenedesmus sp.* With regard to the extraction methods, the Bligh and Dyer (Bligh, 1959) method adapted with the use of ultrasound and chloroform/ethanol solvent systems obtained the higher yield of 19.27% and lower cost of thermal energy when compared to Soxhlet with the same solvent system that resulted in 15.71% of total extracted lipid. The culture mediums containing cassava (5% - 10%) showed better efficiency in the production of dry microalgae biomass compared to the synthetic medium, with the highest productivity of lipids (35.5%) obtained with the microalgae cultivated in the medium supplemented with 10% of cassava. The analysis of the lipid profile allows us to state that the use of cassava as the supplement to the synthetic medium does not affect the quality of fatty acids since they are mostly composed of saturated and monounsaturated fatty acids (76% to 79%), the compounds that guarantee good quality of biodiesel. The obtained results suggest a possibility of using microalgae oil for biodiesel production due to the low unsaturated content which is lower than that of vegetable oils (soybean, cotton, cinnamon).

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