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Growth and Fatty Acid Profile of the *Aspergillus terreus* in Different Culture Media and Temperatures

Crescimento e perfil de ácidos graxos do *Aspergillus terreus* em diferentes meios de cultura e temperaturas

Crecimiento y perfil de ácidos grasos del *Aspergillus terreus* en diferentes medios de cultivo y temperaturas

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Abstract
Fungi are a promising alternative source of oil to produce biodiesel, still very little known. The identification of a species with desirable characteristics is a fundamental component to achieve the economic viability of the process. The study aimed to carry out the evaluation of the fungus *Aspergillus terreus* in different culture media and different temperatures, the production of fungal biomass and in line with obtaining the profile of methyl esters of fatty acids. The fungal biomass revealed that in the NBRIP medium at both a temperature of 29 ºC and 36 ºC, it resulted in a great potential in the production of saturated fatty acids (SFA), which have excellent combustion properties, reaching values of 35.89 and 34,89%, respectively. For most species, the fuel would need to be mixed to make up culture conditions to be optimized and achieve the correct lipid profile, so that the fungal fuel meets European biodiesel production standards (EN 14214). *Aspergillus terreus* from iron ore tailings proved to be a promising microbial biomass as an energy source in the production of biodiesel.
Keywords: Fungus; *Aspergillus terreus*; Biodiesel; Fatty acids; Saturated fatty acids.

Resumo
Os fungos são uma alternativa promissora de óleo para a produção de biodiesel, ainda muito pouco conhecida. A identificação de uma espécie com características desejáveis é um componente fundamental para se obter a viabilidade econômica do processo. O estudo teve como objetivo realizar a avaliação do fungo *Aspergillus terreus* em diferentes meios de cultura e diferentes temperaturas, a produção de biomassa fúngica e em consonância com a obtenção do perfil de ésteres metílicos de ácidos graxos. A biomassa fúngica revelou que no meio NBRIP tanto a uma temperatura de 29 ºC quanto a 36 ºC, resultou em um grande potencial na produção de ácidos graxos saturados (SFA), que apresentam excelentes propriedades de combustão, atingindo valores de 35,89 e 34,89%, respectivamente. Para a maioria das espécies, o combustível precisaria ser misturado para compor as condições de cultura a serem otimizadas e atingir o perfil lipídico correto, de modo que o combustível fúngico atenda aos padrões europeus de produção de biodiesel (EN 14214). *Aspergillus terreus* de rejeitos de minério de ferro provou ser uma biomassa microbiana promissora como fonte de energia na produção de biodiesel.
Palavras-chave: Fungo; *Aspergillus terreus*; Biodiesel; Ácidos graxos; Ácidos graxos saturados.

Resumen
Los hongos son una prometedora fuente alternativa de petróleo para la producción de biodiésel, que aún se conoce muy poco. La identificación de una especie con características deseables es un componente fundamental para lograr la viabilidad económica del proceso. El estudio tuvo como objetivo realizar la evaluación del hongo *Aspergillus terreus* en diferentes medios de cultivo y a diferentes temperaturas, la producción de biomasa fúngica y, en consecuencia, obtener el perfil de ésteres metílicos de ácidos grasos. La biomasa fúngica reveló que en el medio NBRIP tanto a una

temperatura de 29 ºC como de 36 ºC, resultó en un gran potencial en la producción de ácidos grasos saturados (AGS), los cuales tienen excelentes propiedades de combustión, alcanzando valores de 35,89 y 34,89%, respectivamente. Para la mayoría de las especies, el combustible debería mezclarse para componer las condiciones de cultivo a fin de optimizar y lograr el perfil de lípidos correcto para que el combustible fúngico cumpla con las normas europeas para la producción de biodiésel (EN 14214). El *Aspergillus terreus* de los relaves de mineral de hierro demostró ser una biomasa microbiana prometedora como fuente de energía en la producción de biodiésel.
Palabras clave: Hongo; *Aspergillus terreus*; Biodiesel; Ácidos grasos; Ácidos grasos saturados.

1. Introduction

Metric measures of microbial diversity place communities of native fungi as the richest species, with 4,763 fungi being found when submitted to bioinformatics filters (Maron et al., 2018). Fungi have relatively simple nutritional needs, and therefore most species would be able to survive perfectly under aerobic conditions, when supplied with nutrients such as glucose, ammonium salts, inorganic ions and some growth factors (Martín et al., 2019). Vesicular arbuscular mycorrhizal fungi, considered symbionts, are an exception to the one described above, as they require a partner for growth, such as plants for their cultivation (Bholay, Barbosa and Jadhav, 2018).

The elementary requirements for fungal survival are classified into macronutrients, which are provided in millimolar concentrations, and micronutrients, which are provided in micromolar concentrations (Maron et al., 2018). Macronutrients comprise essential sources of carbon, nitrogen, oxygen, sulfur, phosphorus, potassium, and magnesium. Micronutrients, on the other hand, correspond to elements called trace elements such as calcium, copper, iron, manganese, and zinc (Martín et al., 2019).

Some fungi are considered oligotrophic, that is, they can survive in environments with a very limited supply of nutrients, growing in inhospitable environments, with high acidity and at temperatures above 37 ºC, acting in the capture of small amounts of volatile organic compounds of the atmosphere (Velez et al., 2018). Chemotrophs are fungi that need fixed forms of organic compounds, ranging from simple hexoses such as glucose to some types of polysaccharides such as starch and cellulose, as a means of supplying carbon and energy (Meena and Siddhardha, 2019).

The sustainable exploration of this biodiversity has contributed to the discovery of fungi aiming their use as a source of commercially exploitable products, and among the products that can be obtained from this enormous biodiversity are fatty acids, which are important for human health, demonstrating potential in the production of food or pharmaceutical additives, and this is due to their biological activities (Maron et al., 2018; Tonato et al., 2018). Another applicability of fatty acids developed by fungi would be their incorporation in animal feed and possibly also in the production of biodiesel, from the conversion of triacylglycerides into fatty acid methyl esters, called FAME (Sitepu et al., 2019).

Our study aimed to verify two important issues in the cultivation of fungi. First, the influence of nutritional composition and temperature on fungal development in different culture media. Second, the composition of the fatty acid profile in the biomass of the *Aspergillus terreus*.

Table 1: Elementary constitution of the means of enrichment and growth.

Reagents	Formula	Means of Growth %		
		NBRIP	General use	9K-glucose
Glucose	$C_6H_{12}O_6$	1,0	1,0	1,0
Magnesium chloride hexahydrate	$MgCl_2.6H_2O$	0,5	-	-
Magnesium sulfate heptahydrate	$MgSO_4.7H_2O$	0,025	0,1	0,05
Ammonium sulfate	$(NH_4)_2SO_4$	0,01	-	0,3
Calcium nitrate	$Ca(NO_3)_2$	-	-	0,0013
Iron sulfate heptahydrate	$FeSO_4.7H_2O$	-	-	0,001
Potassium chloride	KCl	0,02	-	0,01
Sodium chloride	NaCl	-	0,1	-
Ammonium chloride	NH_4Cl	-	0,5	-
Calcium phosphate	$Ca_5(OH)(PO_4)_3$	0,5	0,5	0,5
pH		7.0 ± 0,2	7,2 ± 0,2	4,5 ± 0,2
Source		Nautiyal, 1999, modified.	Verma et al., 2001, modified.	Silverman e Lundgren, 1959, modified.

Source: Authors.

2. Material and Methods

The experiment was carried out at the Laboratory of Environmental Microbiology and Biotechnology at the Federal University of Goiás - UFG - Campus Goiânia. The following work aims to carry out a quantitative case study, regarding the growth and fatty acid profile of *Aspergillus terreus* in different culture media and temperatures.

2.1 Biological material

The biological material used in this article was the fungus *Aspergillus terreus*, resulting from the isolation of the iron ore tailings sample, as described in the experimental article "Phosphorus solubilization by isolated iron ore tailings fungus" (Ferreira et al., 2020).

2.2 Growth media

The *Aspergillus terreus* was inoculated into three different growth media, as shown in Table 1 (Nautiyal, 1999 modified, Verma et al., 2001 modified, Silverman e Lundgren, 1959 modified. All media were dissolved in distilled water and sterilized at 121 ºC for 20 minutes.

2.3 Biomass production

For the production of fungal biomass, three 4 mm fragments of the microbial mycelium, cultivated in Petri dishes containing PDA at 30 ± 2 ºC for 7 days, were added to Erlenmeyers containing the three culture media, shown in Table 1, in triplicate, submitted separately at temperatures of 29 ºC and later at 36 ºC. For visual evaluation, the media were incubated and removed at times zero, 72 hours and 168 hours, submitted to 130 rpm in the incubator. After each period, a visual assessment of the means was made, and a photograph was taken. Then, the media from the 168 hours were vacuum filtered, separating the biomass from the liquid medium. The biomass of the media was dried in a desiccator and placed in Eppendorf®, to carry out an analytical test of fatty acid composition. To perform the fatty acid composition in fungal biomass, the samples were identified as A1. A2 and A3, equivalent to growth in NBRIP, general purpose and 9K-glucose media, respectively, at a temperature of 29 ºC, and being done in the same way for samples B1, B2 and B3, but at a temperature of 36 ºC.

2.4 Scanning Electron Microscopy (SEM)

The pellets obtained from the NBRIP medium after 168 hour were analyzed via SEM in a JEOL microscope (JSM 6610), equipped with EDS Thermo scientific NSS special imaging. The samples were subjected to special preparation techniques, such as fixation in glutaraldehyde, dehydration in ascending concentrations of acetone, drying by the Autosamdri® CO_2 critical point, mounting the sample in "Stub", and coating of gold films on the sample by the system of evaporation known as "sputtering" (Denton Vaccum, Desk V), for subsequent conduction in the SEM, aiming at performing external morphological observations of *Aspergillus terreus* and images capturing.

2.5 Transmission Electron Microscopy (TEM)

Fungal fragments were collected in pellet format, referring to a period of 168 hours in the NBRIP medium, for analysis by TEM in a JEOL microscope (JEM 2100) operated with 100 KeV, equipped with EDS Thermo Scientific. For TEM, the samples were subjected to special preparation techniques, such as fixation of the material in glutaraldehyde, post-fixation in osmium tetroxide, dehydration in ascending concentrations of acetone, infiltration in resin, and finally polymerization for subsequent conduction in TEM, aiming to carry out internal morphological observations of *Aspergillus terreus* and images capture.

2.6 Direct Transesterification (DT)

The direct transesterification procedure of fungal biomass was performed using the Hartman & Lago method adapted to the microscale, as described by Soares et al. (2014). The upper content, containing FAME, was collected, and analyzed by gas chromatography. To calculate the content of esters in the biomass, 1.0 mL was removed from the heptane phase, obtained via DT, and transferred to an Eppendorf® flask, previously tared. The samples were left in a desiccator for solvent evaporation until the FAME mass reached constant weight. The ester content was obtained by the relationship between the FAME mass and the initial biomass mass used in the DT procedure.

2.7 Fatty acid composition

The composition of fatty acids in the form of methyl esters was made by gas chromatography (HRGC-FID) using the Agilent 7890 equipped with a flame ionization detector (FID) and a split/splitless injector to analyze the composition of the FAME. The capillary column was DB-WAX (30m x 0.25mm x 0.25 µm). The initial temperature of the oven was 70 ºC, being heated at a rate of 10 ºC min-1 to 240 ºC and maintained at this temperature for 13 minutes. Heated again at a rate of 5 °C min^{-1} to 250 °C. The injector temperature was maintained at 310 °C in split mode with a ratio of 10:1 and an injection volume of 2 µL. The detector was kept at 310 ºC. Hydrogen 5.0 was used as carrier gas at a linear velocity of 42 cm s^{-1}, and nitrogen 5.0 was used as auxiliary gas at a rate of 20 mL min^{-1}.

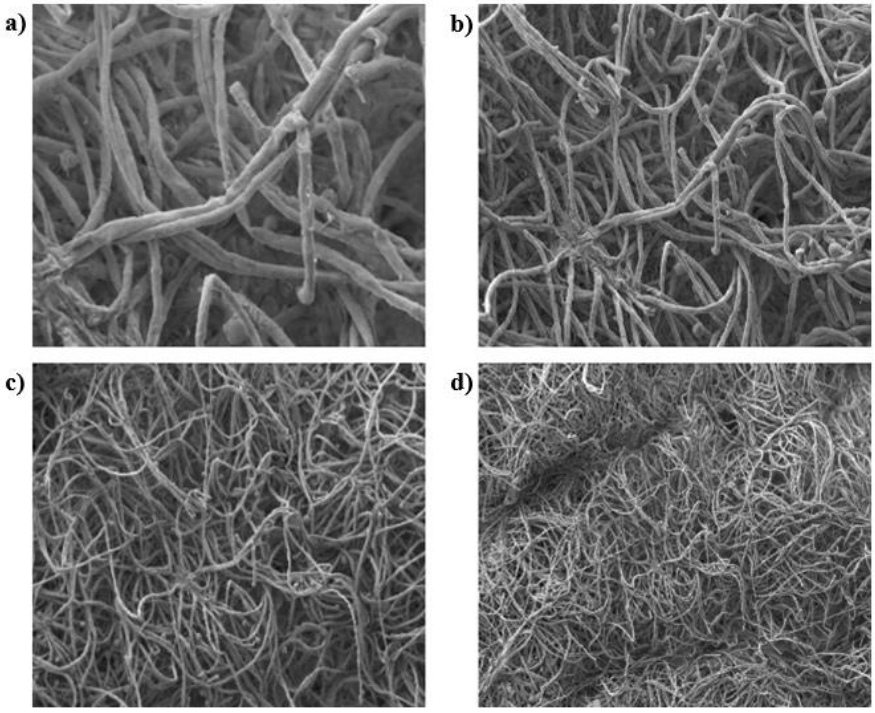
The FAMEs were identified by comparison with the retention times of samples of known composition as soybean oil, peanuts and crambe (*Crambe abyssinica*) by analyzing the FAME reference standards (Nu-Check-Prep®) by gas chromatograph (HRGC-MS) Shimadzu 17A coupled to a Shimadzu QP 5050 mass spectrometer, interfaced at 280 °C. Helium was used as a carrier gas at 38 cm s^{-1}. The operating conditions for the oven, injector and column were the same as those used for HRGC-FID. Each sample was injected three times by HRGC-FID and HRGC-MS.

3. Results and Discussion

The images obtained by SEM, show microphotographic images of the external structure of *Aspergillus terreus*, attributing observations of the morphological and structural aspects of the fungal mycelium, as shown in Figure 1, revealed the display of many filamentous structures of variable size. It is also possible to observe the presence of sporangia in development among the filaments, responsible for promoting the fixation and perpetuation of the fungal species.

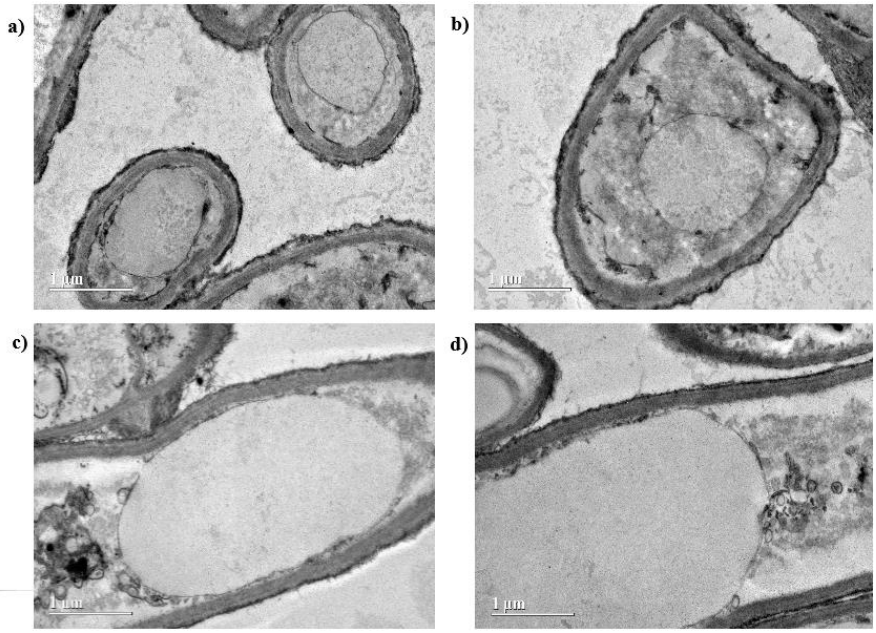
In the images produced by TEM, they bring microphotographic observations of the internal structure of *Aspergillus terreus*, showing morphological and dispersion observations of fungal samples, as shown in Figure 2. The images show the presence of apparently “empty” structures, with globular or globular structures. oval, without the perception of the presence of apparent internal structures, which stand out with the presence of a well-developed vacuolar system.

Figure 1: SEM image capture aiming to perform external morphological observations of the fungus, from the NBRIP medium for a period of 168 hours. Images with fungus resolution of (a) x2000 and 10 µm, (b) x1000 and 10 µm, (c) x500 and 50 µm, and (d) x250 and 100 µm.



Source: Authors.

Figure 2: TEM image capture aiming to perform internal morphological observations of the fungus, from the NBRIP medium for a period of 168 hours. Images with resolution of the fungus of x5000 and 1 µm.



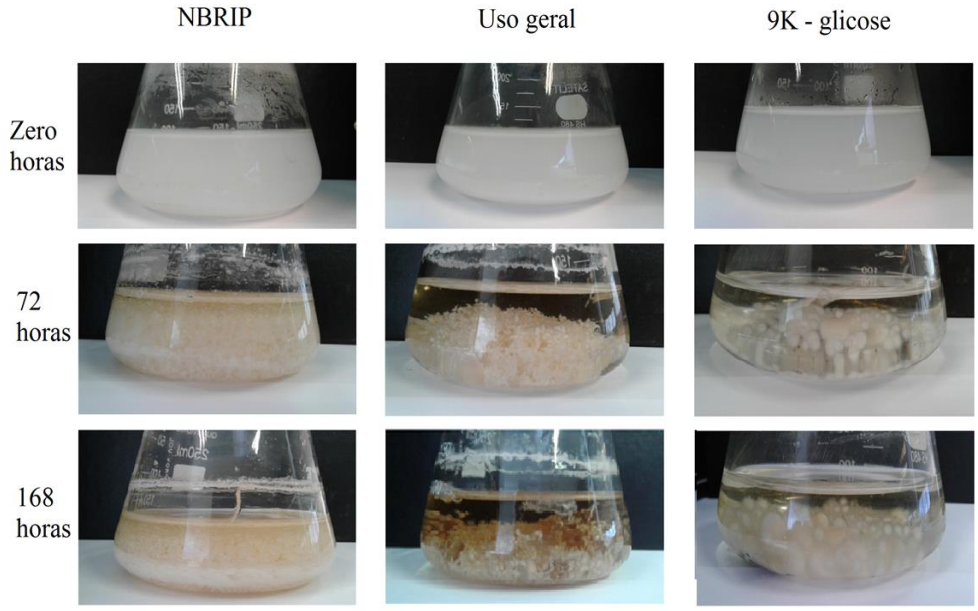
Source: Authors.

The NBRIP and 9K-glucose culture media have the greatest nutritional composition, as shown in Table 1, with each nutrient being responsible for the fungal cell's vital functionality. According to Walker and White (2018), the macronutrients carbon (C), nitrogen (N), oxygen (O), sulfur (S), phosphorus (P), potassium (K), magnesium (Mg), and the micronutrients

calcium (Ca), iron (Fe), which make up the culture media shown in Table 1, are necessary for the cell growth of the fungus. Carbon (present in glucose) has the structural function of the fungal cell when in combination with hydrogen, oxygen and nitrogen, they act as a source of energy. Nitrogen (present in NH_4^+ salts, urea, and amino acids) acts structurally and functionally on proteins and enzymes. Oxygen (present in the air, O_2), works as a substrate for respiratory oxidative enzymes and other mixed functions, being essential for ergosterol and the synthesis of unsaturated fatty acids. Sulfur (present in sulfates and methionines), is available for the composition of sulfur amino acids and vitamins. Phosphorus (present in phosphates), acts in energy transduction, composing the nucleic acid and the membrane structure. Potassium (present in K^+ salts), is responsible for the ionic balance of the cell, acting in the enzymatic activity. Magnesium (present in Mg^{2+} salts), acts in the enzymatic activity, constituting the cell structure and organelles. Calcium (present in Ca^{2+} salts), being responsible as a second messenger in signal transduction; and iron (present from ferric salts, Fe^{3+} chelation by siderophores and Fe^{2+} release inside the cell) is contained in a small hemoprotein, called cytochrome, which is responsible for carrying out electron transport.

The NBRIP and general use culture media showed great potential in the development of fungal biomass, respectively in this order, at a temperature of 29 ºC in relation to the other culture media, as shown in Figure 3. This condition was favorable due to the elementary nutritional constitution of the two culture media, which have components in common in greater amounts than other media such as Mg^{2+} and NH_4^+ salts that act exclusively on the structure and functionally in the fungal cell, in addition to the presence of a sugar (glucose) having carbon as an essential element for the supply of energy. The pH around 7 also contributed efficiently to the production of fungal biomass only for the NBRIP culture media and general use, although most of the fungi have their optimal growth in acid pH.

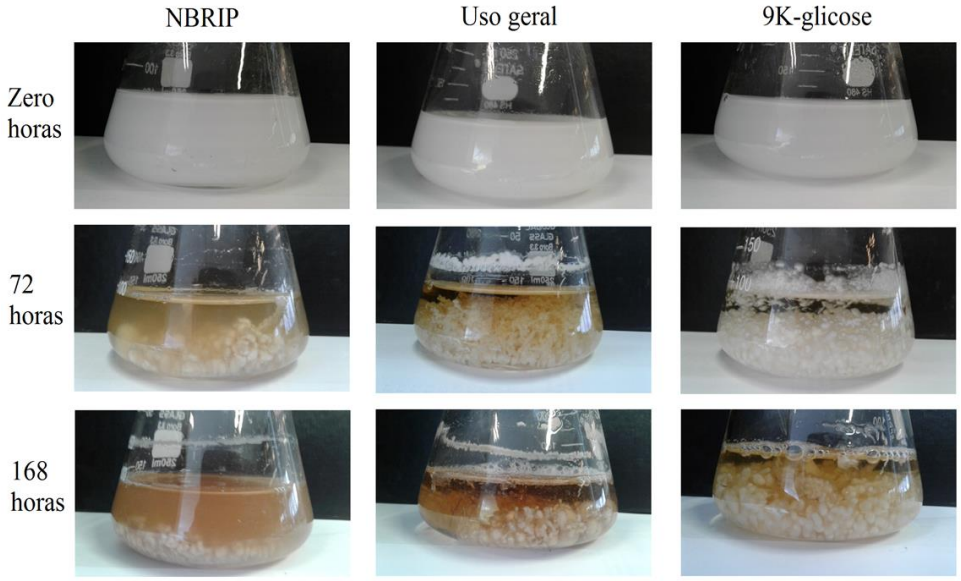
Figure 3: Profile of culture media (NBRIP, general use, 9K-glucose and 9K), submitted to three incubation periods (zero, 72 and 168 hours) at a temperature of 29 º C.



Source: Authors.

However, the 9K-glucose medium showed great affinity for growth and biomass production at a temperature of 36 ºC, when compared to other culture media (Figure 4). Because the 9K-glucose medium presents a considerable inhospitable condition for microbial development, due to its high temperature and acidic pH, it was evident that the fungus showed satisfactory potential for biomass development, when compared to NBRIP and general purpose media.

Figure 4: Profile of culture media (NBRIP, general purpose and 9K-glucose), subjected to three incubation periods (zero, 72 and 168 hours), at a temperature of 36 º C.



Source: Authors.

When comparing the content of fungal biomass produced between NBRIP media at 29 ºC and 9K-glucose at 36 ºC, the two media were quite similar, with greater emphasis on the NBRIP media, due to its greater volume in fungal biomass content, with pellets Spherical smaller and more dispersed in the reaction medium, compared to the 9K-glucose medium, the pellets present a larger spherical shape dispersed in the reaction medium.

Sixteen fatty acid methyl esters were identified in *Aspergillus terreus* samples, as shown in Table 2, ranging from C14:0 to C24:0, and according to Figure 3 o C16:0, C18:0, C18:1 cis9 and C18:2 cis9, 12, that is, the fatty acids hexadecanoic (palmitic), octadecenoic (stearic), cis-9-octadecenoic (oleic) and cis-9,12-octadecadienoic (linoleic), respectively, are considered to be methyl esters majority. These methyl esters, in the NBRIP medium at 29 ºC and at 36 ºC, represent 93.27% and 96.63%, respectively, the total constitution of fatty acids in the fungus, now, in the general purpose medium at 29 ºC and at 36 ºC, is 96.05% and 94.49%, in due order, represents the total content of fatty acids in the fungus, whereas in the 9K-glucose medium at 29 ºC and at 36 ºC, it is represented by 95.97% and 95.20%, in this order, the total constitution of fatty acids in fungal biomass.

Table 2: Contents of fatty acid methyl esters profile in fungal samples.

Fatty acid methyl esters		Contents (%) ± dp					
		A1	A2	A3	B1	B2	B3
C14:0	Tetradecanoic	0,25 ± 0,03	0,16 ± 0,01	0,33 ± 0,01	0,22 ± 0,00	0,51 ± 0,09	0,25 ± 0,00
C15:0	Pentadecanoic	0,18 ± 0,00	0,25 ± 0,00	0,21 ± 0,00	0,18 ± 0,00	0,35 ± 0,06	0,26 ± 0,00
C16:0	Hexadecanoic	15,36 ± 0,18	14,04 ± 0,12	17,79 ± 0,16	16,36 ± 0,50	18,06 ± 0,85	17,76 ± 0,01
C16:1 cis7	cis-7-Hexadecenoic	0,21 ± 0,02	0,52 ± 0,01	0,82 ± 0,01	0,19 ± 0,00	0,95 ± 0,01	0,52 ± 0,00
C17:0	Heptadecanoic	0,51 ± 0,01	0,49 ± 0,00	0,22 ± 0,00	0,53 ± 0,00	0,33 ± 0,02	0,51 ± 0,00
C17:1 cis7	cis-7-Heptadecanoic	0,11 ± 0,01	0,24 ± 0,00	0,15 ± 0,00	0,12 ± 0,01	0,22 ± 0,02	0,25 ± 0,01
C18:0	Octadecennoic	15,01 ± 0,33	6,92 ± 0,01	6,10 ± 0,02	15,72 ± 0,01	4,73 ± 0,24	7,12 ± 0,00
C18:1 cis9	cis-9-Octadecenoic	34,27 ± 0,58	46,85 ± 0,10	47,06 ± 0,10	35,26 ± 0,21	38,62 ± 0,29	42,74 ± 0,10
C18:2 cis9,12	cis-9,12-Octadecadienoic	28,63 ± 0,21	28,24 ± 0,01	25,02 ± 0,09	29,19 ± 0,19	33,08 ± 0,04	27,58 ± 0,03
C18:3 cis9,12,15	cis-9,12,15-Octadecatrienoic	0,18 ± 0,00	0,28 ± 0,00	0,32 ± 0,01	0,18 ± 0,00	1,31 ± 1,11	0,07 ± 0,00
C19:0	Nonadecanoic	2,87 ± 0,45	0,07 ± 0,04	0,29 ± 0,21	0,11 ± 0,04	0,06 ± 0,02	0,57 ± 0,12
C20:0	Eicosanoic	0,75 ± 0,02	0,48 ± 0,00	0,34 ± 0,00	0,77 ± 0,00	0,32 ± 0,02	0,59 ± 0,00
C20:1 cis11	cis-11-Eicosanoic	0,10 ± 0,02	0,29 ± 0,01	0,30 ± 0,01	0,09 ± 0,01	0,23 ± 0,03	0,21 ± 0,00
C20:2 cis11,14	cis-11,14-Eicosanoic	0,10 ± 0,03	0,17 ± 0,00	0,21 ± 0,00	0,21 ± 0,12	0,22 ± 0,05	0,16 ± 0,00
C22:0	Docosanoic	0,40 ± 0,02	0,35 ± 0,01	0,22 ± 0,01	0,36 ± 0,01	0,31 ± 0,06	0,42 ± 0,01
C24:0	Tetracosanoic	0,45 ± 0,01	0,65 ± 0,01	0,62 ± 0,01	0,52 ± 0 02	0,71 ± 0,18	0,98 ± 0,01
ΣSFA		35,89 ± 0,12	23,65 ± 0,02	26,27 ± 0,04	34,89 ± 0,06	25,60 ± 0,17	28,71 ± 0,01
ΣMUFA		34,58 ± 0,16	47,66 ± 0,03	48,18 ± 0,03	35,54 ± 0,06	39,80 ± 0,09	42,70 ± 0,03
ΣDUFA		28,73 ± 0,12	28,41 ± 0,01	25,23 ± 0,05	29,40 ± 0,16	33,30 ± 0,05	27,74 ± 0,02
ΣTUFA		0,18 ± 0,00	0,28 ± 0,00	0,32 ± 0,01	0,18 ± 0,00	1,31 ± 1,11	0,07 ± 0,00
ΣUFA		64,11 ± 0,12	76,35 ± 0,02	73,73 ± 0,03	65,11 ± 0,08	74,40 ± 0,22	71,29 ± 0,02

dp - standard deviation; Σ – summation; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; DUFA – di-unsaturated fatty acids; TUFA – tri-unsaturated fatty acids; UFA – unsaturated fatty acids. A1 and B1 - NBRIP medium; A2 and B2 – general purpose medium; and A3 and B3 - 9K-glucose medium. Source: Authors.

C16:0 showed a higher content of fatty acids in the 9K-glucose culture medium, with a value of 18.06% of methyl ester, at a temperature of 36 ºC, and the C18:0, showed a higher productive performance of the order of 15 .72% in the NBRIP medium at the same temperature, that is, at 36 ºC, whereas the C18:1 cis9 reached a productivity of 47.06% in the 9K-glucose medium, at the temperature of 29 ºC, while the C18 methyl ester: 2 cis9, 12, with a value of 33.08% in the general purpose environment, at a temperature of 36 ºC.

Considering the culture media at a temperature of 29 ºC, both the NBRIP medium, with the methyl esters C18:0 and C18:2 cis9, 12, and the 9K-glucose medium with the methyl esters C16:0 and C18:1 cis9 demonstrated higher yields in the production of fatty acids. Now, observing the culture media at a temperature of 36 ºC, the general purpose medium obtained greater yield in the production of methyl esters for C16:0 and C18:2 cis9, 12, however the fatty acids C18:0 and C18:1 cis9 showed higher yields in NBRIP and 9K-glucose media, respectively.

When observing the temperature, the methyl esters C16:0, C18:0 and C18:2 cis9, 12, at a temperature of 36 ºC, corresponding to the general purpose media, NBRIP and 9K-glucose, respectively, confirm the highest yields in production of fatty acids, while at 29 ºC, the C18:1 cis9 in the 9K-glucose medium was the only one that exhibited greater efficiency in the production of fatty acid.

A correlation between the culture media and the culture temperatures of the filamentous fungus, the NBRIP and 9K-glucose media and the temperature of 36 ºC were the ideal conditions for the highest yield in the production of fatty acid methyl esters. Among the two culture media, the 9K-glucose medium was the one that performed better with the greatest efficiency in the production of fatty acids, revealing that in the acid condition of 4.5 ± 0.2, the filamentous fungus performs the most productive effectiveness of fatty acids.

The samples showed up to 1.31% tri-unsaturated fatty acids (TUFA) and absence of polyunsaturated fatty acids (PUFA), which are values lower than those described in the European standard EN 14214, which qualifies the FAME content for biodiesel, which defined the maximum limit of 12% for TUFA and 1% for PUFA (EUROPEAN UNION, 2010). Such determination is justified by the fact that such unsaturated fatty acids classified as TUFA and PUFA provide low oxidative stability to the biofuel, despite improving the flow properties (Knothe, 2005). While SFA have very good combustion properties, they can cause cold flow problems (Jeong et al., 2008). Therefore, it is noticeable that the fungal samples studied appear to have great potential as biomass to be used as raw material to produce biodiesel

The FAME evaluation revealed that the *Aspergillus terreus* samples had higher contents of unsaturated fatty acids (64.11 to 76.35%) at 29 ºC and (65.11 to 74.40%) at 36 ºC than saturated (23) .65 to 35.89%) at 29°C and (25.60 to 34.89%) at 36°C. Among the culture media used in the study, the NBRIP medium revealed both at a temperature of 29 ºC and at 36 ºC, great potential in the production of saturated fatty acids, reaching values of 35.89 and 34.89%, respectively. Palmitic (C16:0) and stearic (18:0) fatty acids emerged as the main saturated fatty acids in fungal biomass in NBRIP medium at 29 ºC, with values of 15.36 and 15.01%, respectively each, revealing as possible biomass and physical and chemical conditions capable of producing biodiesel.

4. Conclusion

The fungal biomass of *Aspergillus terreus* showed greater production efficiency of saturated fatty acids in the NBRIP medium when the FAME profile was observed both at a temperature of 29 ºC and at a temperature of 36 ºC, with a small percentage of SFA being observed in the latter. The acidic pH was also shown to be a major factor in the efficiency of production of acidic compounds, such as fatty acids, thus creating conditions for the development and perpetuation of the

species in an adverse environment. Thus, even if we do not have any process for optimizing the production of fungal biomass in the production of biodiesel, it is still possible to produce such a biofuel of interest and perhaps in similar or even greater quantities than conventional sources.

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