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Influence of the Different Carbon and Nitrogen Sources on the Production of Biodiesel by Oleaginous Fungi *Aspergillus terreus*, *Aspergillus fumigatus*

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Abstract:

In the present study, the growth and total lipid contents of two oleaginous fungal isolates *Aspergillus terreus*, *Aspergillus fumigatus* were compared in different nitrogen and organic carbon sources. Artificially the fungi were cultured on media consisting of various mono- or di- or polysaccharides and peptone or yeast extract as elementary sources for carbon and nitrogen, respectively. Media containing sucrose /yeast extract or glucose/ yeast extract were the most effective for lipid production from fungal, during two weeks incubation period, the highest biomass of dry weight was (19.6 , 18.8) g / L , (25.8 , 30.5) g /L and lipid yield (1, 0.97)g/L, (0.65, 0.65) g/ L for two isolates *Aspergillus terreus* and *Aspergillus fumigatus* respectively then followed maltose/ yeast extract then starch / yeast extract. Analysis of fatty acid produced by two fungal isolates using Gas Chromatography showed the existence of a wide range of fatty acids, these fatty acids were together saturated and unsaturated. The results revealed that the unevenness in the fatty acids composition mainly depends on the type of carbon and nitrogen sources. The existence of saturated and mono saturated fatty acids in *A.fumigatus*, *A. terreus* prove that they are good candidate for biodiesel production.

Key words: Biodiesel, Carbon sources, Nitrogen sources, Oleaginous fungi.

Introduction:

Many governmental and industrial efforts are looking to find alternative fossil fuels that are renewable and carbon free. One of these alternative is biodiesel derived from microorganisms like algae, bacteria and fungi (1). However, little information has been documented about the lipids utilization from bacteria and fungi for the production of biodiesel (2). Fungi are a good source of lipids used in biodiesel production (3).

Oleaginous microorganisms accumulate lipids Single Cell Oils(S.C.Os) such as yeasts, fungi, bacteria and micro-algae, with more than 20% of their cell mass being consisting of lipids, have appeared as a potential feedstock for biodiesel production (2, 4).

There are numerous factors which hearten the use of fungi for production of lipid. For example, the accumulation of lipid in oleaginous fungi has been observed when an essential element in the medium (such as the nitrogen source) comes

to be inadequate and the carbon source becomes an addition (5) .

The nitrogen in oleaginous species is favorably directed toward the synthesis of lipid, through the Triacylglycerol (TAG) accumulation that occurs within intracellular lipid bodies (6). The nitrogen depletion in oleaginous species plays a key role in stimulating the accumulation of lipid in *Mucor circinelloides* and *Gliocladium roseum* which was done at conditions of stressed like high glucose -to yeast extract ratio and elevation temperature (7, 8).

Several species of molds and yeasts could accumulate substantial amounts of intra-cellular lipid (9) The production of lipid can be adjusted by adding essential nutrients to culture media and/or by changing culture growth conditions (10). Carbon and nitrogen sources have the effect on the biomass and production of lipid in oleaginous fungi. (11) found glucose in syrup of date and sucrose in liquid

medium is most suitable for the biomass growth of *A. terreus* and lipid production.

present study focuses on two oleaginous fungal isolates and optimizes medium conditions in different carbon and nitrogen sources for increasing the activity of oleaginous fungi for the production of biodiesel.

Materials and Methods

Two oleaginous fungal isolates *A. terreus*, *A. fumigatus* were obtained from Department of Biology, College of Science., Mustansiriyah university, Baghdad, Iraq which are isolated from different Iraqi environments then cultured on potato dextrose agar at 28° C for 5 days .

Utilizing Different Carbon sources for Lipid Production

Different carbon types were used as a sole source of carbon in basal medium : monosaccharide (glucose, sucrose) disaccharides (maltose) and polysaccharides (starch) the medium prepared by dissolving 20g yeast extract,150g carbon source type in 1000ml distilled water and the medium was sterilized in autoclave for 20 minute under 15 lbs/In² pressure at 121°C.

Utilizing Nitrogen source for Lipid Production

Pepton and yeast extract were used as nitrogen source in basal medium both alone. Dissolving 20g type of nitrogen source ,150 g sucrose in 1000ml distilled water and the medium was sterilized in autoclave for 20 minute under 15 lbs/In² pressure at 121°C.

Determination of the Biomass for Oleaginous Fungi

The two oleaginous fungal isolates (*A. terreus*, *A. fumigatus*) were cultured in basal medium each 500ml of medium was set in one liter Erlenmeyer bottle and supplemented with five discs from fungal inoculum in diameter 5 mm for each fungal isolate at pH 6.0 and incubated for 14 days at 30 °C, then the fungal growth was noticed on 14th day. The Biomass was gained using No.2 filter paper (whatman) inside a cabinet for biological safety. The biomass collected was twice washed with water (distilled water) and for 24 h dried at 60 °C or till the weight became constant then the dry weight was esteemed gravimetrically mg /L (12).

Extraction of Fungal Lipid

The lipids were extracted from dried biomass by using solvent system methanol 2:1(v/v) chloroform: methanol. The isolates samples of

fungal dried were smashed with mortar and pestle by consecutively adding (5ml) of methanol and (10ml) of chloroform then 8ml of this blend was pulled out and added to one gram of biomass then vortexed for 5 minutes. The solution prepared from normal saline containing (NaCl (7.3g) dissolved in 10ml of water) was pulled out (2ml) then added for each tube after being vortexed for 5 min . At 3000 rpm for 15 minutes. The sample tubes were centrifuged and the lower layer of NaCl, water and methanol was excluded by pipette. The remaining of solvent that was dried then the percentage of the cell dry weight to extracted lipids were assessed gravimetrically in mg /L (8).

Analysis by Gas Chromatography and Biodiesel Production

The total lipids from dried biomass in the previous item were extracted. The compositions of fatty acid of the produced lipid by isolates of fungi that were extracted from the previous items were determined by depending upon the method of (13) (analysis of fatty acid methyl esters (FAMES)) with some change. By trans-esterification reaction, the FAMES were produced. 2.5% V/V H₂SO₄/CH₃OH and 2 ml of methanol was added as a catalyst to 100 mg of the crude lipid. The reaction was proceeded at 90 C for 45 min (water bath). Then, 2 mL n-hexane and 1 mL H₂O were added. The Fatty Acid Methyl Esters were liquefied into the n-hexane. The solution at 2000 rpm was submitted to centrifugation for 15 min to get FAMES within the compact the water from the hexane phase , then transmitted into ampoules made glass by using pipettes of Pasteur. The fatty acid methyl esters in n-hexane were examined after adding 0.1ml of solution (methanol 1% W/ V, 1ml of heptane and KOH,) to the Fatty Acid Methyl Esters by using a gas chromatograph. The weight proportions of the fatty acids were analyzed, a gas chromatography was used to (GC) analyzer (made in Kyoto, Japan GC-17A model by Shimadzu Inc.,) with the Chromatography Data Management system (Advantech Inc., Taipei, Taiwan), (6890N, Agilent, Japan) equipped with an supelco wax tm 10 capillary column (Agilent, Japan, length 30 m 0.32 mm I.D, 0.25 Mm flim thickness, substance fused silica) and a flame ionized detector (FID). The functional settings were as follows : temperature of oven 180 C , and helium air were used as carrier gas. The detector temperatures 260 C and the injector temperatures 250 C .

Results and Discussion

Biomass and total lipid yields

The yield of lipid and the highest biomass of *A. terreus* isolate were determined, after 14 days of growth in liquid medium under the different carbon (glucose, maltose, sucrose, starch) and rich, nitrogen source (yeast extract). The results indicated a quantitative biomass. The highest value of the dry weight of biomass was 19.6 g/l, 18.8 g/l to carbon sources sucrose and glucose respectively and the lowest value was 15.2 g/l at starch carbon source, while for the medium culture which consisted of carbon source, sucrose and pepton as nitrogen source the total biomass recorded 8.0 g/L (Fig. 1).

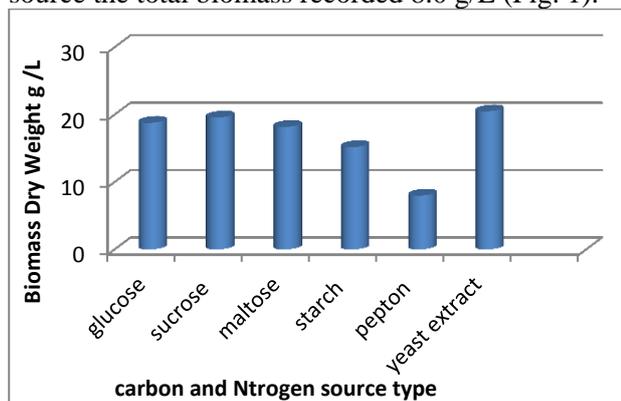


Figure 1. Dry Weight of fungal biomass g/l of *A. terreus* in basal medium at different Carbon and Nitrogen sources, pH adjacent to 7 and incubated at 120 rpm at 30 °C for 14 days.

The lipids extracted from biomass was estimated. The maximum harvest of lipids was 1 g/L by using sucrose as source of carbon and yeast extract as source of nitrogen 0.9 g/L (Fig. 2).

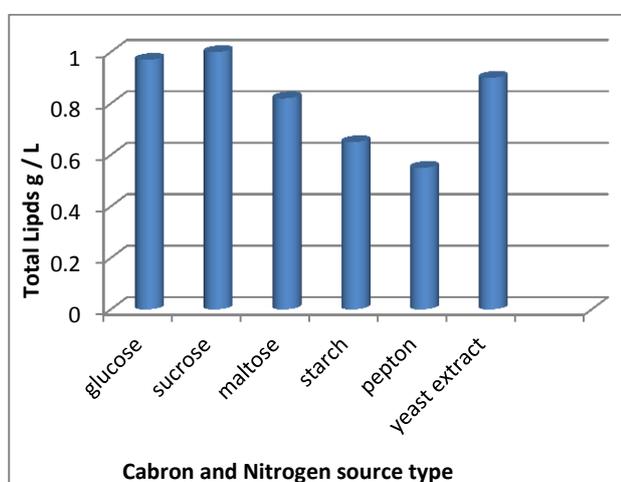


Figure 2. Total Lipid (g/L) of *A. terreus* in basal medium at different Carbon and Nitrogen sources, pH adjacent to 7 and incubated at 120 rpm at 30 °C for 14 days.

While The best biomass Productivity, *A. fumigatus* showed the optimum conditions in liquid medium enriched with maltose as carbon source. The dry weight of biomass recorded the highest value at 30.5 g/L and 26.7 g/L with glucose and yeast extract respectively as nitrogen source as seen in Fig. 3.

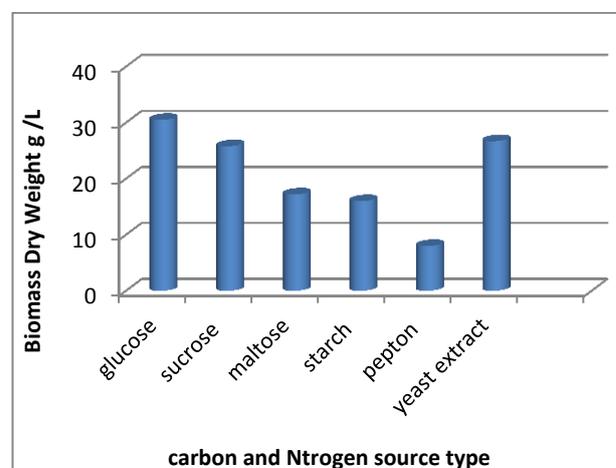


Figure 3. Dry Weight of fungal biomass g/l of *A. fumigatus* in basal medium at different Carbon and Nitrogen sources, pH adjacent to 7 and incubated at 120 rpm at 30 °C for 14 days.

However the highest value of lipid extracted from liquid medium was enriched with carbon source sucrose 1 g/L and yeast extract 0.9 g/L as nitrogen source as shown in Fig. 4.

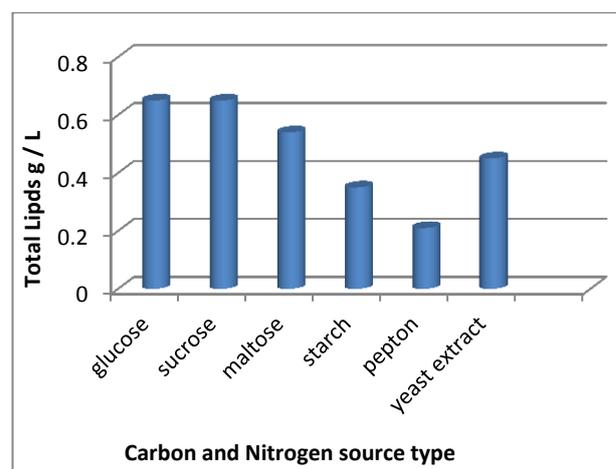


Figure 4. Total Lipid (g/L) of *A. fumigatus* in basal medium at different Carbon and Nitrogen sources, pH adjacent to 7 and incubated at 120 rpm at 30 °C for 14 days.

Among the various carbon sources tested, the disaccharides sucrose (consist from two glucose molecules) and monosacchride glucose were the best organic carbon source for *A. fumigatus* and *A. terreus* and yeast extract the better nitrogen source

for biomass and total lipid yield in comparison to other organic carbon and nitrogen sources. The most commonly carbon source employed for growing fungal oleaginous and lipid production is the glucose. Moreover, the fatty acids composition of the lipids was affected by the concentration of initial glucose. These results agree with other studies around getting lipids for the production of biodiesel from a number oleaginous fungi isolates specially *Aspergillus* spp. (14, 15, 16, 11).

The production of Biodiesel and Gas Chromatography -GC analysis

The extraction of lipids for two fungal isolates to fatty acid methyl esters were detected by GC -Gas Chromatography. As exposed in Table 1 and 2, the profiles of fatty acid by Gas Chromatography with RT (Retention Time)

revealed the presence of Oleic acid and Palmitic acid mostly in two isolates selected in different carbon sources: glucose, sucrose, maltose and starch which provided the maximum concentrations between the others kinds of Stearic acid, fatty acids, Linolenic. Palmitic acid, or hexadecanoic acid, is the most common fatty acid (saturated) and it is a main constituent of the biodiesel (17).

Mainly, fungal oils contained higher fraction of saturated fatty acids like palmitic, stearic and myristic acid than unsaturated fatty acids oleic and linoleic acid in the fatty acid methyl esters analysis. The biodiesel quality is dependent on the fatty acid profile of the oil used as feedstock for its production. Therefore, the production of biodiesel, the lipids obtained should be analyzed and compared with standard specifications for renewable diesel (18, 19).

Table 1. The composition of fatty acid extracted from total lipids of *A. terreus* in different carbon sources

(FA)	Percentage of the total lipids extracted on different carbon and nitrogen sources (yeast extract)					Rotation Time
	Glucose	Sucrose	Maltose	Starch	Yeast extract	
Palmitic C16	28.8925	15.26		17.7618	25.34	4.71
Stearic C18	-	-	-	3.5526		8.49
Oleic C18.1	71.1074	43.00	63.58	40.5170	72.73	8.897
Linolenic C18.2	-	38.73	-	38.1642	3.031	10.39

Table 2. The composition of fatty acid extracted from total lipids of *A. fumigatus* in different carbon sources

(FA)	Percentage of the total lipids extracted on different carbon and nitrogen sources (yeast extract)					Rotation Time
	Glucose	Sucrose	Maltose	Starch	Yeast extract	
Palmitic C16	15.4150	15.26	11.0609	12.7588	25.34	4.71
Stearic C18	-	-	-	53.692	-	8.49
Oleic C18.1	42.7277	43.00	47.3328	-	72.73	8.897
Linolenic C18.2	41.8572	38.73	-	33.9719	3.031	10.39

Conclusion:

The results of present study reveal that the Yeast Extract Sucrose (YES) medium was the best medium for growth and lipid production of oleaginous fungal isolates *Aspergillus terreus*, *Aspergillus fumigatus*. The hydrocarbon profile of mycodiesel extracted from oleaginous fungi have number of compounds normally associated with diesel fuel which containing saturated more than unsaturated fatty acids

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in AL-Mustansiriyah University.

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اثر المصادر الكربونية والنتروجينية المختلفه على انتاج الديزل الحيوي من قبل الفطريات المنتجه للدهون *Aspergillus fumigatus* و *Aspergillus terreus*

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الخلاصة:

في الدراسة الحاليه تمت المقارنه بين تاثير مصادر كربونيه ونتروجينيه مختلفه على النمو ومحتوى الدهون في عزلتين من الفطريات المنتجه للدهون *Aspergillus fumigatus* و *Aspergillus terreus* . زرعت العزلتين الفطريه على اوساط زرعيه حاويه على مصادر كربونيه مختلفه السكريات الاحاديه والثنائيه والمتعدده ومصادر نتروجينيه متضمنه البيبتون وخالصة الخميرة وكانت الاوساط الحاويه على السكروز / خالصة الخميره او السكروز / خالصة الخميره اكثر تاثيرا على انتاجيه الدهون للعزلتين الفطريه خلال فترة حضانه اسبوعين حيث بلغت اعلى قيمة للكتلة الحيويه المتمثلة بالوزن الجاف (18.8 , 19.6) , (30.5 , 25.8) غم / لتر ونسبة الدهون (1 , 0.65) , (0.65 , 0.97) g/L / لتر لكلا العزلتين الفطريه *Aspergillus fumigatus* و *Aspergillus terreus* على الترتيب ثم تبعتها اوساط الغنيه بالمالتوز/ خالصة الخميرة واخيرا النشا / خالصة الخميرة . وفيما يخص تحليل الحوامض الدهنيه بواسطه جهاز كروموتوكرافيا الغاز Gas Chromatography والمنتجه من قبل كلا العزلتين الفطريتين لوحظ وجود انواع مختلفه تضمنت Palmitic , stearic acid , Oleic acids و linoleic acid اي المشبعه والغير مشبعه. اظهرت النتائج تفاوت في تركيب الحوامض الدهنيه يعتمد على نوع مصادر الكربون والنتروجين ، وجود الحوامض الدهنيه المشبعه الاحاديه والغير مشبعه في كلا الفطرين *A. terreus* و *A.fumigatus*. اثبتت كونهما مرشحان جيدين في انتاج الديزل الحيوي.

الكلمات المفتاحية : الديزل الحيوي ، الفطريات المنتجه للدهون ، مصادر الكربون ، مصادر النتروجين