PRODUCTION OF SINGLE CELL OIL FROM LOCAL FUNGAL ISOLATE

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ABSTRACT

Twenty fungal isolates have been isolated from different sources, soils(Some of them were contaminated with mineral oils) wheat, rice bran and rotting sesame seeds. Five isolates contained several highly lipidic particles. The highest lipid amount (5.95 g/L) was obtained from *Mucor* sp. that isolated from soil which was contaminated with mineral oils and the oil yield was 50% of biomass. The results indicated that all carbon resources gave more than 20% lipid. The best carbon source for lipid production was Glucose and then Xylose which gave 50% and 46% oil respectively. The best sources of nitrogen were yeast extract and NH_4Cl which were achieved 54% and 50% oil respectively. Regarding the incubation period, it has been noticed that a temperature range 20 - 30 C ° was favorable for oil production and the highest percentage (55.5%) was obtained at 25 C°. The optimum pH for lipid production was 6.5, it gave 56% oil. The optimum condition for lipid production in respect to size of inoculum, agitation rate, and incubation duration were 1 X 10^7 spore/ml, 150 rpm, 120h respectively. This condition achieved 56.5%, 58% and 68% oil respectively.

Key words: Oleaginous Fungi, oil, polyunsaturated fatty acids

انتاج الزيت وحيد الخلية من عزلة فطر محلية

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المستخلص:

تم الحصول على 20 عزلة فطرية من مصادر عزل مختلفة ، تميزت 5 منها بأحتوائها على العديد من الاجسام الحاوية على الزيت، مع ملاحظة تفوق العزلة *Mucor*.sp المعزولة من تربة ملوثة بزيوت معدنية/بغداد في كمية الزيت المنتجة والبالغة 5.95 غم/لتر تقريبا وبنسبة مئوية وصلت الى 50% تقريبا. اوضحت النتائج المستحصل عليها بأن جميع مصادر الكاربون المستعملة اعطت نسبة مئوية للزيت المنتج اعلى من 20% مع افضلية للكلوكوز يليه الزايلوز اذ بلغت النسبة المئوية للزيت المنتج 50% و 46% تقريبا على التوالي، مع ملاحظة افضلية مستخلص الخميرة وكلوريد الامونيوم NH_4Cl مصدرا للنتروجين بالمقارنة مع مستخلص الخميرة وكبريتات الامونيوم $(NH_4)_2SO_4$)، اذ بلغت النسبة المئوية التقريبية للزيت المنتج 54% و 50% على التوالي. اوضحت النتائج ان المدى الحراري مابين 20– 30 م° اعطى نسبة مئوية جيدة للزيت المنتج مع الحصول على اعلى نسبة عند الحضن بدرجة حرارة 25 م والبالغة تقريبا 55.5%، واعلى نسبة مئوية تم الحصول عليها عند الرقم الهيدروجيني 6.5 لوسط الانتاج والبالغة 56% ، في حين لوحظ ان اعلى نسبة مئوية للانتاج تم الحصول عليها عند حجم لقاح 1×10^7 خلية/مل وعدد دورات 150دورة/ دقيقة ومدة حضن 120 ساعة، اذ بلغت 56.5% و58% و68% تقريبا على التوالي.

الكلمات المفتاحية: الفطريات الزيتية، زيت، الاحماض الدهنية الطويلة المتعددة غير المشبعة

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INTRODUCTION

Single cell oils(sco) are the fat which is obtained from microorganisms. These oils are similar to that from plant and animal sources qualitatively and structurally (15). El-Naggar *et al*. (7) mentioned that the increase in using oils for industrial and food purpose led to focusing on finding new microbial sources. The microorganisms have the ability to produce oils which have advanced features compared with soybean, Palm and Sun flower oils. That is because it contains fatty acids which could not be synthesized by plants or animals such as Gamma-linolenic-acid GLA, arachidonic acid (ARA) and docosahexaenoic acid (DHA) (10)..Other researchers (15) mentioned that ARA and DHA which exist in fungi oils are very important for human brain growth. Moreover, it can be added to some types of baby milk because it increases growth rate. Also, there are many of physiological functions which are encouraged using it widely in food, medical, chemical and cosmetics fields. Several researchers (6) mentioned that polyunsaturated fatty acids have significantly commercial importance for preventing atherosclerosis, heart disease and make in- appropriate conditions for the growth of cancer cells. Furthermore, the researcher mentioned that the composition of the culture media has a evident effect on oil accumulation inside the microorganism cells.. The aim of this study is to get fungal isolates from local sources which produce a large amounts of single cell oils and identifying the optimal production conditions.

MATERIALS AND METHODS

Samples collections and fungi isolation:

Samples were collected from different soures being, soils(Some of them were contaminated with mineral oils), wheat, rice bran and rotting sesame seeds. The Pan *et al.* method (14) was adapted for fungi isolation using medium containg glycerol 100g/l, $(NH_4)2SO_4$ 1g/l, KH_2PO_4 1g/l, $MgSO_4$. 7H2O 0.5g/l, yeast extract 0.2 g/l, chloramphenicol 100mg/l. The pH adjusted to 5.5 and autoclaved at121°C/15 pounds / inch^2 for 15 min. One gram of each sample was added to Erlenmeyer flasks containing 250 ml of above mentioned medium. All flasks were incubated at 30°C for 96 h / 180 rpm/min. at the end of the incubation, 0.5 ml from each flask was transferred to petri dish then the media which contain glucose 20g/l, $(NH_4)_2SO_4$ 2g/l, KH_2PO_4 0.5g/l, $MgSO_4$. $7H_2O$ 0.2g/l, $CaCl_2.2H_2O$ 0.1 g/l, chloramphenicol 100mg/l and Agar 20 g was added to each dish. All petri dishes were incubated at 28°C for 3 days. The transfering process was repeated for several times to get pure isolates (17).

Qualitative screening

The method of Enshaeich *et al* (8) was applied in this experiment using Sudan blank B. The pure isolates were kept in PDA media for further experments.

Quantitative screening

After preparing the spores suspention, by harvesting spores from cultures *mucor* sp. grown for 5 days on PDA slants at 30C$^\circ$ by scraping the agar surface with 200 ml sterile water, the spores were calculated using Hemocytometer. The limited nitrogen media was used for quantitative screening contained glucose 40g/l, $(NH_4)_2SO_4$ 2g/l, KH_2PO_4 7 g/l, 2 g, $MgSO_4$. $7H_2O$ 1.5 g/l, yeast extract 1 g/l. All inoculated flasks were incubated at 30°C using shaking incubater (200 rpm/min) for 7 days.

Lipid Extraction

Biomass separation was conducted using Whatman NO.1 under vacuum. The Biomass washed many times using sterilized distilled water, then dried at 40C°and the weight was recorded. Bligh and Dyer method (6) was used for extraction of lipids from the renewable biomass. The obtained quantity was estimated using the equation of other researchers (12) (14). Sco productivity (Lipid content) = Sco weight / Cell dry weight X 100. The isolate which produced the highest amount of lipid has been selected.

Determination of the optimum conditions for the Lipids production

The same media as in production media was used with some modification in the quantitative screening to determine the optimum conditions.

Determination of the optimum carbon source: same concentration of carbon sources as in production media were used including sucrose, fructose, lactose and Xylose, individually.

Determination of the optimum nitrogen source: The organic nitrogen sources were replaced by another organic sources being Peptone , beef and malt extracts. Moreover, the inorganic source in the media was replaced by ammonium chloride.

Temperature: The inoculated flasks were incubated at the temperature ranged from 10 to 45°C.

pH: The media pH was adjusted by (0.5 N)NaOH or (0.5 N)HCl to achieve pH values ranged from 2.5 to 10.5.

Size of inoculum: Different size of inuculum were used being, ($1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$,$1x10^9$) spore / ml.

Agetation and stirring: All flasks were incubated in shaker incubators using different rpm, being 100, 150, 200, 250, 300 rpm.

Incubation time: All flasks were incubated for different periodes being (24, 48, 72, 96, 120, 144, 168 hrs). The results were recorded.

RESULTS AND DISCUSSION

The fungal isolates: Table 1 shows the fungal isolates(20 isolate) which were obtained from different sources. The diagnostic process depending on cultural and morphological properties revealed that eight of the fungal isolates belong to *Aspergillus* sp. and two isolates belong to *Fusarium* sp.. Five of the isolates back to *Mucor* sp. and three of the isolates belong to *Rhizopus* sp. Two isolates belong to *Penicillium* sp.

Table 1. The fungal isolates and their sources

Fungal Isolates	The Fungal Sources	Number
***Aspergillus* sp.**	**University of Baghdad, College of Agriculture**	**5**
***Aspergillus* sp.**	**Soil is contaminated with mineral oils / Baghdad**	**3**
***Fusarium* sp.**	**Rice bran**	**2**
***Mucor* sp.**	**Soil is contaminated with mineral oils / Baghdad**	**2**
***Mucor* sp.**	**Rice bran**	**3**
***Rhizopus* sp.**	**Wheat bran**	**2**
***Rhizopus* sp.**	**Soil is contaminated with mineral oils / Baghdad**	**1**
***Penicillium*sp**	**Rotting sesame seeds**	**2**

Qualitative screening for fungal isolates: Sudan Black B method was used. five of studied isolates contained many oil bodies(Table 2), hence these five isolates been selected to conduct the quantitative screening for fungal isolates.

Table 2.The fungal isolates which tested microscopy which contain several oil bodies

Fungal Isolates Name	Fungal Isolates Source
***Mucor* sp.**	**Soil is contaminated with mineral oils / Baghdad**
***Mucor* sp.**	**Rice bran**
***Penicillium* sp.**	**Rotting sesame seeds**
***Fusarium*sp**	**Rice bran**
***Aspergillus* sp.**	**Soil is contaminated with mineral oils / Baghdad**

Quantitative screening for fungal isolates: Table 3 shows the results of the quantitative screening for the five isolates which selected from the qualitative screening. The results indicated that the highest amount of oil was 5.95 g/l(50% of biomass) produced by *Mucor* sp. which isolated from the soil that contaminated with mineral oils. Based on this result, this isolate was selected to carry out the next experiment in this study. As shown in table 3, all isolates of oily type contain more than 20% (of Biomass) oil. Li and otheres (13) was mentioned that the oleaginous microorganisms such as yeasts and molds can produce a large amounts of oil under the appropriate culture conditions. Many researchers (2) stated that the microorganisms which produces more than 20% oil of its biomass were called Oleaginou

Table 3. The Quantitative screening for the fungal isolates which contain more than 20%(of Biomass) oil

Fungal Isolates	Biomass weight	Total fat weight	Oil percentage (%)
***Mucor* sp.**	**11.2**	**5.95**	**50**
***Mucor* sp.**	**10.8**	**4.92**	**49**
***Penicillium* sp.**	**13.0**	**3.0**	**24**
***Fusarium*sp**	**11**	**3.5**	**29**
***Aspergillus* sp.**	**17**	**4.2**	**24.7**

The optimum carbon source: Figure1 shows that Glucose was the optimum carbon source to produce the single cell lipids. The total percentage of lipid was 50% of dried weigh *Mucor* sp. which isolated from the soil that contaminated with mineral oils. Then, xylose as carbon source the percentage was reached to 46% while the percentage decreased when other carbon sources were used. That is because the fungi grow quickly on simple sugars. Kaboosi and Behbahani(12) were stated that the carbon source plays a major role in the fat accumlation process. Also, other researcher mentioned that different types of carbohydrates, which are derived from agro-industrial waste are used for SCO production, Such as sucrose, molasses, hydrolyzed starch products, fructose, whey, in addition to natural hydrolysates such as tomato waste and rice straw hydrolysate, also alcohols such as hexadecanol. Chatzifragkou and others (6) was mentioned that SCO production in presence of glucose was more than that with lactose, starch and pectin respectively, because the α_amylase, β_galactosidase and polygalactoronase enzymes are not sufficient for metabolic activities.

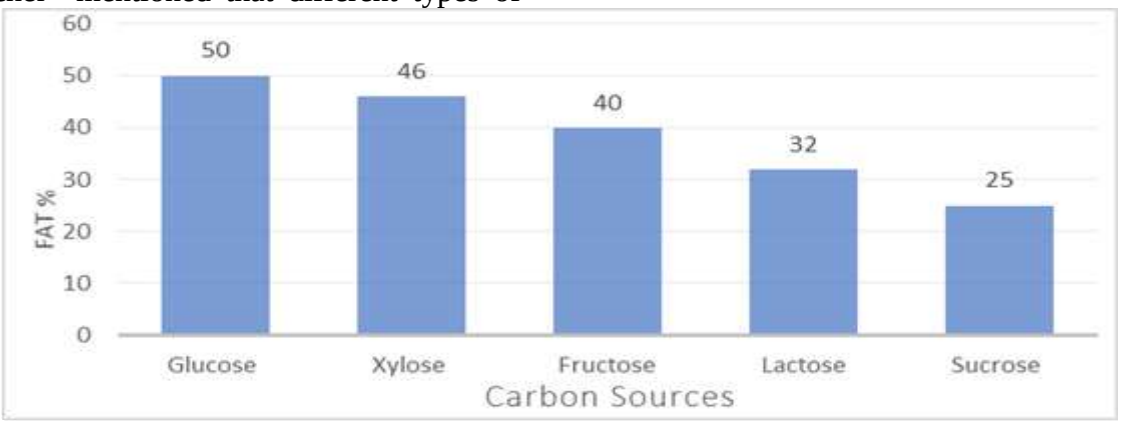

Figure 1. Effect of the carbon source on the percentage of the single cell oil which produced from *Mucor* sp

The optimum nitrogen source: Figure 2 has shown that the yeast extract with ammonium chloride as nitrogen source gave the highest percentage (54%) for lipid production as compared with that of the yeast extract with ammonium sulfate (50%). The percentages of lipid produced using peptone, meat extract and malt extract were 48%, 44% and 40%, respectively, these results could be attributed to the difference in the nature and solubility of above mentioned nitrogen soures (5) . Kaboosi and Behbahani (11) was reported that the nitrogen sources were effective in lipid accumulation such as NH_4Cl, Yeast extract, asparagine glutamate and ammonium tartrate. The best source for nitrogen was the yeast extract. Also, the researchers mentioned to the possibility of replacing yeast extract by corn steep liquor in lipid production for commercial production using fungi

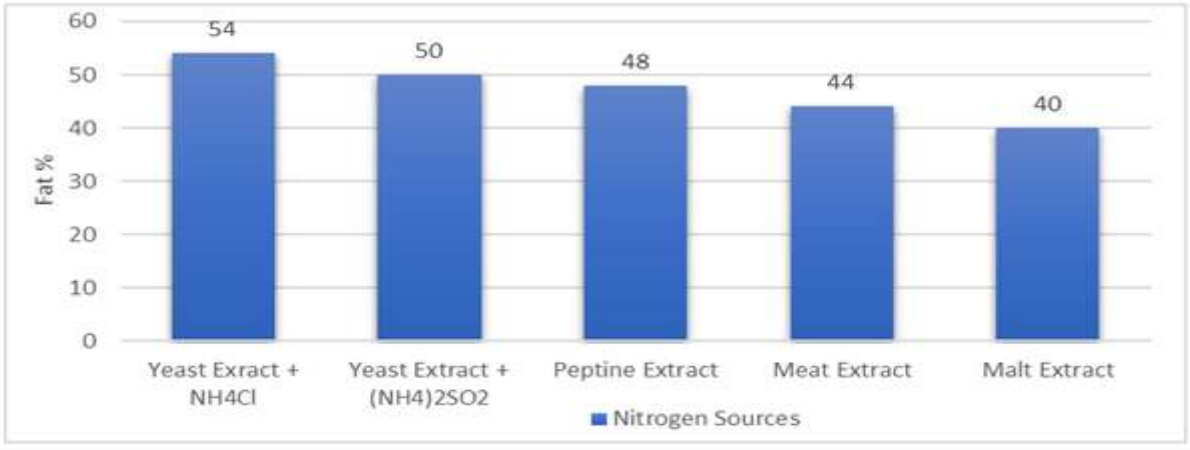

Figure 2. Effect of the nitrogen source on the percentage of the single cell oil which produced from *Mucor* sp

The Optimum Temperature : Figure 3 shows, that the highest production (55.5%.) of single cell oil was obtained at 25 °C as optimum incubation temperature. It has been noticed that single cell oil at 20 °C- 30 °C were also high and reached to 45 - 50 % respectively.

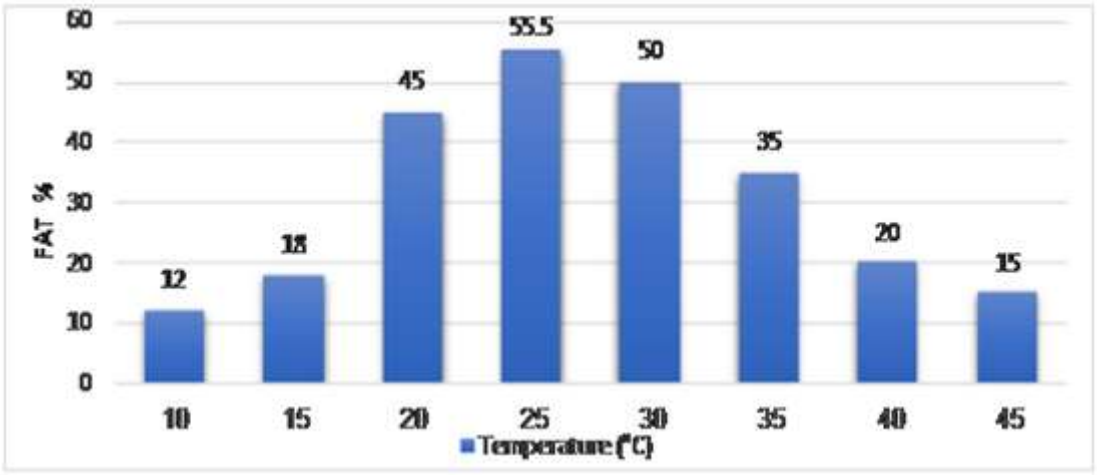

Figure 3. Effect of the temperature on the percentage of the single cell oil which produced from *Mucor* sp

The Optimum pH

Figure 4 indicated that pH range from 5.5 to 7 was appropriate for single cell lipid production from *Mucor* sp. The highest percentage was obtained at pH 6.5 which was 56%. There was no growth at pH 2.5. The best pH for single cell lipid production from *Candida albicans* NRRL-Y-12983 and *LipomycesStarkeyi*NRR-y-11557 was 6 using corn gluten meal (7) . Enshaeich and others (8) indicated that the best pH for single cell lipid production from *Rodotoruls* 11 was 6.5 it has been noticed that the production of single cell oil at pH 5.5-7.5 were also high.

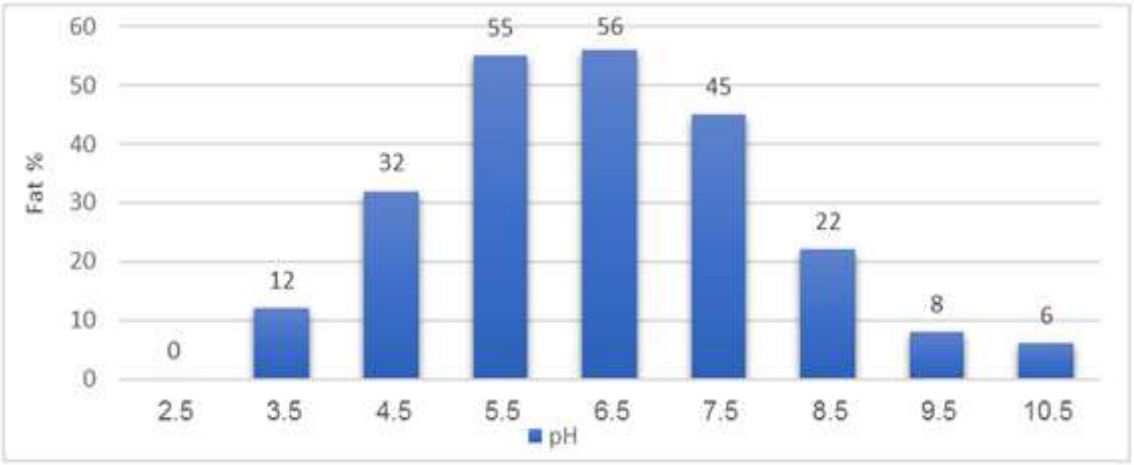

Figure 4. Effect of pH on the percentage of the single cell oil which produced by *Mucor*sp. at 25°C

The size of the inoculum: The results in figure 5 indicated that the highest lipid production 56.5% was obtained at inoculum size $1X10^7$ spore/ml. The percentage was decreased when the size of a inoculum was more or less than $1X10^7$ spore/ml. That is because of using inoculums size more than $1X10^7$ spore/ml causes competition on nutrients which give negative reflect on the oil production

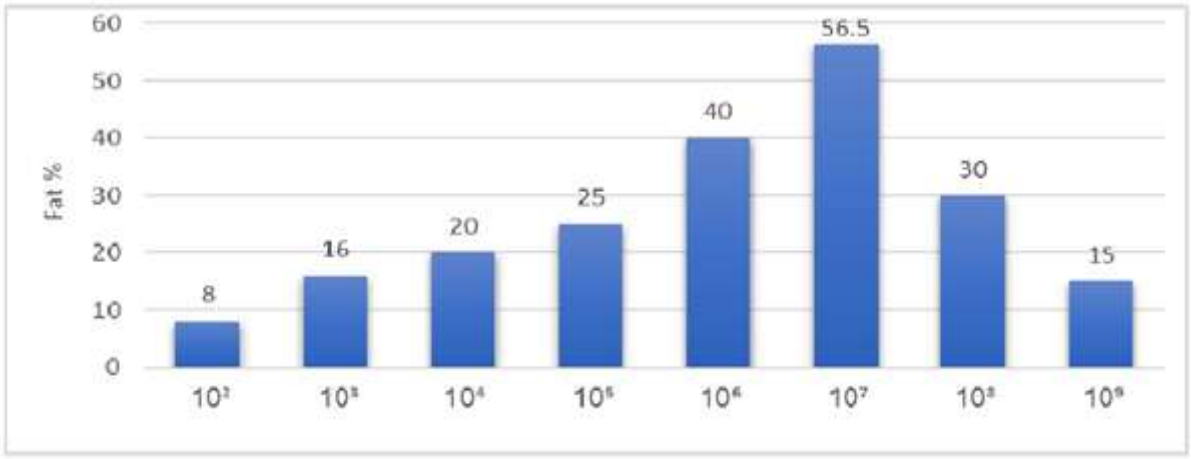

Figure 5. Effect of the size of inoculum on the percentage of the single cell oil which produced by *Mucor* sp. at 25°C and pH 6

Agitation and stirring: The ventilation and stirring is one of the important factor for single cell lipid production, because most of the oleaginous microorganisms are aerobic. Figure 6 shows that the best percentage gained at 150 rpm which was 58% of Biomass.

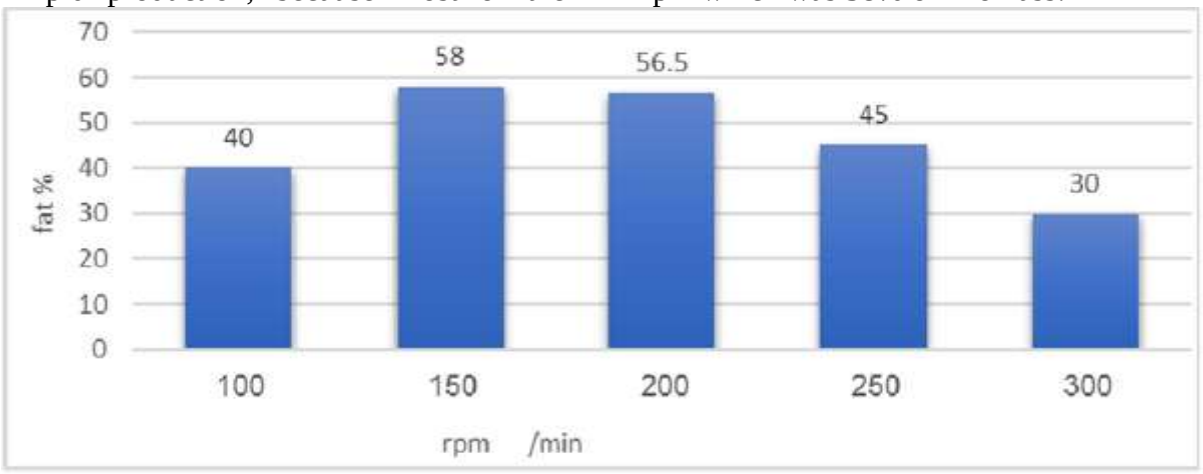

Figure 6. Effect of the Agitation speed the percentage of the single cell oil which produced by *Mucor* sp. at 25°C and pH 6.5

Incubation time: Figure 7 shows the effect of incubation time on single cell lipid production. The highest percentage was obtained upon 120 hours of incubation (5 days) at 25 °C which was 68%. This lipid amount is similar to the amount which was obtained after 144 and 168 hours incubation. These results indicate that the carbon source was exhausted from media after 5 days of incubation.

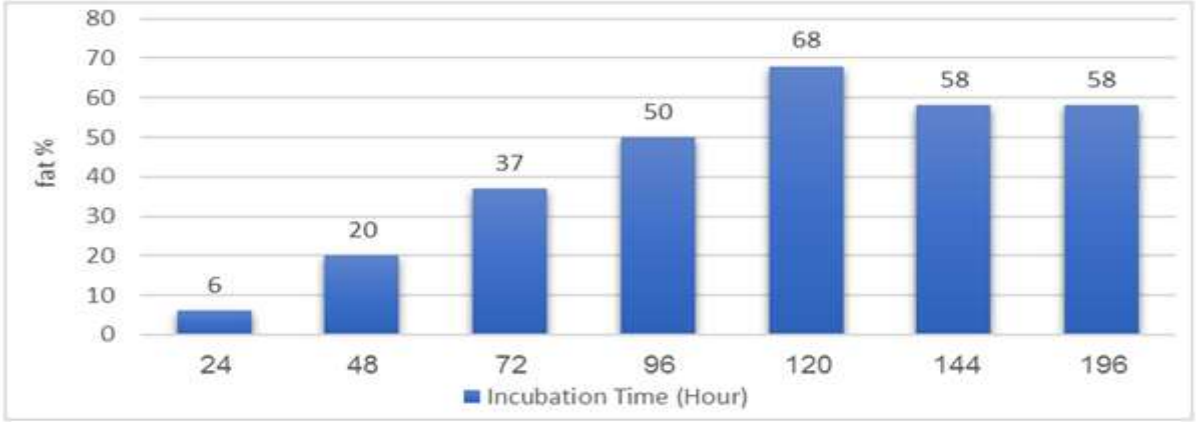

Figure 7. Effect of the incubation timeon the percentage of the single cell oil which produced by *Mucor* sp. at 25°C, pH 6.5 and 150 rpm/min

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