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Single Cell Oil Production by an Oleaginous Yeast Strain in a Low Cost Cultivation Medium

[1]Husain A. El-Fadaly, [2]Noura El-Ahmady El-Naggar and [3]El-Sayed M. Marwan
[1]Department of Microbiology,
Damietta Faculty of Agriculture Mansoura University, Damietta, Egypt
[2]Department of Bioprocess Development,
Genetic Engineering and Biotechnology Research Institute,
Mubarak City for Scientific Research and Technology Applications,
Alexandria, Egypt
[3]Department of Pharmacognocy, Faculty of Pharmacy,
Mansoura University, Mansoura, Egypt

Abstract: An oleaginous yeast strain, *Cryptococcus curvatus* NRRLY-1511 was used for the production of single cell oil (SCO) using a low cost cultivation medium containing beet molasses and corn gluten meal as carbon and nitrogen sources. Obtained results showed that 125 and 0.130 g L^{-1} showed to be the optimum concentrations for carbon and nitrogen, respectively. In addition, 28°C, 72 h, 5.5, 200 rpm were the favorable values of growth temperature, incubation period, pH value of cultivation medium and agitation speed, respectively. The extracted lipids were mainly 30.68% linoleic acid (C18:2), 22.66% oleic acid (C18:1) and 16.74% palmitic acid (C_{16}:0). Furthermore, the GC analysis also showed that the total saturated fatty acids (n = 9) represented 41.96% while the value of the total unsaturated fatty acids (n = 6) was 58.04%. These results giving possibility to use such this yeast strain to produce SCO in a low cost medium from economic point of view.

Key words: Single cell oil production, oleaginous yeast, low cost medium, single cell protein, gas chromatography

INTRODUCTION

The concept of single cell oil (SCO) produced by lipid-producing (oleaginous) microorganisms as the supplementary sources of conventional oils and fats has attracted attention since the early 1980s. The majority of those lipids in these organisms are triacylglycerols (TAG), containing long-chain fatty acids that are comparable to conventional plant oils. It would be important to develop new oil resources by using microbes, which offers many advantages compared with traditional methods using animal fat and plant oils. Oleaginous yeasts are often considered for the production of single cell oil. The economics of these bioprocesses has become more favourable when zero or negative value waste substrates are utilized as carbon or nitrogen sources (Pan *et al*., 2009). The efficiency of oil

Corresponding Author: Noura El-Ahmady Ali El-Naggar, Department of Bioprocess Development, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research and Technology Applications, Alexandria, Egypt

biosynthesis by yeast and its composition depend on the genetic properties of the yeast strains, cultivation conditions and the composition of culture medium. Lipids are important storage compounds in plants, animal and fungi. Storage lipids are usually found within special organelles knows as lipid particles or lipid bodies. In yeast, these lipid bodies accumulate during stationary phase and they can constitute up to 70% of the total lipid content of the cell (Zweytick *et al*., 2000).

Triacylglycerols (TAGs) and steryl esters (STEs) are the most important storage lipids of eukaryotes cells such like yeast cells. TAG provides an energy source on one hand and a source of fatty acids for membrane phospholipid formation on the other hand. Mobilization of STE sets sterols free, which are also required for membrane proliferation, especially of the plasma membrane. In the yeast as in other eukaryotic cells, TAG and STE form the core of the so-called lipid particles which are surrounded by a phospholipid monolayer with a small amount of proteins embedded (Sorger *et al*., 2004).

The polyunsaturated fatty acids, linoleic (C18:2) and linolenic (C18:3) are the essential fatty acids. They are not synthesized by the human body and, therefore, must be obtained from the dietary intake of foods containing them. Linoleic acid aids in the prevention of platelet aggregation leading to blood clotting in the blood vessels as described by Renaud (1990). Linoleic acid, as essential fatty acid is able to reduce the incidence of cardiac arrest in rats induced by artery occlusion resulting in an increased blood flow to the heart, reduces serum cholesterol. They may prevent the development of cardiovascular disease (Hansen and Chiu, 2005). In addition, palmitic acid (C16:0) and oleic acid (C18:1) have a neutral effect on serum cholesterol, when the dietary intake of cholesterol is low.

The aim of this study was to determine the optimal conditions for oil biosynthesis by an oleaginous yeast strain, *Cryptococcus curvatus* NRRLY-1511 using a low cost cultivation medium containing beet molasses and corn gluten meal as carbon and nitrogen sources.

MATERIALS AND METHODS

Yeast Strain and Cultivation Media

The yeast strain used in this investigation was *Cryptococcus curvatus* NRRLY-1511. The cultivation medium of Lindberg and Molin (1993) was used for fermentation and cultivation of the yeast strain. Yeast extract malt broth (YMB) medium was used for inoculum preparation and maintenance of the used yeast strain.

By-Products Used

Sugar Beet Molasses (SBM) that obtained from sugar beet factory of Belkas, Dakahlia Governorate, Egypt was used as a sole carbon source. This by-product containing about 48% total sugars before purification that became 23.3% after purification. Corn gluten meal obtained from starch and glucose factory as a by-product containing 2.22% nitrogen was used as a sole nitrogen source. Each of these wastes was replaced with the other source in the cultivation medium of Lindberg and Molin (1993) in the same ratio.

Preparation of the Industrial By-Products

Sample of Sugar Beet Molasses (SBM) was prepared as carbon source by diluting with water in an equal volume using the method of Pandey and Agarwal (1993) with little modification. H_2SO_4 solution was used to reduce the pH value to reach 3.0. Sample was boiled at 100°C for 1 h then maintained at room temperature for 24 h, centrifuged at 3000 rpm and filtered. Filtrated solution was used to determine the total sugars to be used as a sole

carbon source. Nitrogenous by-product namely corn gluten meal, was oven dried at 105°C for 2 h and milled. About 7.5 g of waste was add to 100 mL of 1.5% H_2SO_4 and autoclaved for 45 min then filtered. The obtained supernatant was attained at pH 6, being used as a sole nitrogen source.

Preparation of the Inoculum

The inoculum used in the experiments was prepared in Erlenmeyer flasks using YMB medium. The cultivation conditions were carried out at 28°C; pH 5; 72 h and shake speed was 200 rpm. The inoculum size of 3% v/v (5×10^5cfu mL^{-1}) was added to the tested cultivation medium.

Medium Optimization

Fermentation was carried out in 250 mL capacity Erlenmeyer flasks containing 100 mL of the previously mentioned fermentation medium and initial pH was adjusted to 6.0 using a pH meter before autoclaving at 121°C for 20 min then inoculated with 3% v/v (5×10^5 cfu mL^{-1}) of cells suspension of the examined yeast in sterile distilled water. The culture was incubated at the growth temperature on a rotary shaker at 200 rpm for the required fermentation period using LAB-line instrument, Inc., plaza, Mel Rose, Park, ILL. 60160.

Four agro-industrial by-products as carbon sources namely sugar beet molasses, sugar cane molasses, potatoes peel and tomatoes peel were individually added to the basal cultivation medium at a concentration of 10% as glucose to study the effect of each carbon source on the production of microbial oil. Yeast strain was grown on the oil production medium provided with different concentrations of sugar beet molasses ranging from 75-175 g L^{-1} were used to select the optimal concentration.

Determination of Yeast Dry Weight

Yeast dry weight (single cell protein) determination was performed by harvesting culture samples, centrifuged at 5000 rpm, washed twice with distilled water and dried at 60°C to constant weight (Granger *et al*., 1993). The growth yield efficiency (economic coefficient) was calculated according to the following equation:

$$\text{Growth yield efficiency} = \frac{\text{Cell dry weight (g L}^{-1})}{\text{Sugar consumed (g L}^{-1})}\times 100$$

The productivity of oil produced (conversion coefficient) was also calculated according to the following equation:

$$\text{Single cell oil productivity} = \frac{\text{Single cell oil weight (g L}^{-1})}{\text{Cell dry weight (g L}^{-1})}\times 100$$

Determination of Total Sugars

Total sugars were determined according to the method of Herbert *et al*. (1971) as follow: Into a thick walled tube, 1 mL of the tested sample was pipetted and well mixed with 1 mL of 5% phenol solution and then 5 mL of concentrated sulphuric acid were directly added on the surface of a liquid with shaking. The tubes were allowed to stand in a water bath at 25°C for 20 min before reading the density of obtained colour at 490 nm using a Jenway model 6305 UV/visible range spectrophotometer. The standard was carried out by the same process using glucose. The total sugars was expressed as mg glucose/mL using the equation of $Y = 0.0274\ x+0.024$ with $R^2 = 0.9586$.

Single Cell Oil Extraction

Single cell oil extraction was carried out according to the method of Granger *et al.* (1992) as follows: Yeast cells were separated by centrifugation at 6000 rpm (Type 16000, sponnung 220 V, German Democratic Republic) for 15 min and dried at 60°C to constant weight. The dried cells were then milled for 20 min with carbonium powder and extracted using a condenser unit at 60-70°C then filtered using a filter paper Whatman No. 1 and oven dried at 70°C. The single cell oil yield efficiency was also calculated according to the following equation:

$$\text{Single cell oil yield efficiency} = \frac{\text{Single cell oil weight (g L}^{-1})}{\text{Sugar consumed (g L}^{-1})} \times 100$$

Extraction of Fatty Acids

The method of extraction for fatty acids determination of obtained single cell oil was carried out according to the method recommended by Radwan (1978). Extraction was obtained by adding 2.5 mL of 1% sulfuric acid in anhydrous methanol and 1 mL benzene in a sealed tube and heating in a hot water bath at 90°C for 90 min. The tube was allowed to cool, then 4 mL of distilled water and 2.5 mL petroleum ether were added and shaked well. The ether layer (upper layer) was removed in a small vial and evaporated and then 50 mL of n-hexane was added.

Gas Chromatographic Conditions

Sample was injected into a Shimadzu Model GC-8A gas Chromatograph equipped with a Flame Ionization Detector (FID) and a 2.5 M x 3 mm glass column under the following conditions, 5% DEGS coated on 80-100 mesh with chromosorb WHP). The flame ionization detector temperature was 270°C and the column set was temperature programmed from 150 to 180°C at 2°C min^{-1} The carrier gas was nitrogen, with a flow rate of 20 mL min^{-1}; hydrogen flow rate was 75 mL min^{-1} and chart speed 2.5 mm min^{-1} The hydrogen and air flow-rates were 75 and 0.5 mL min^{-1}, respectively. The FID sensitivity was16×10^{2}. Peaks of fatty acids were identified by cochromatography with the standards and relative retention times (Radwan, 1978).

RESULTS AND DISCUSSION

Carbon Source

Four of agro-industrial by-products used as carbon sources were individually examined for single cell oil production by the tested yeast *Cryptococcus curvatus* NRRLY-1511 namely, sugar cane molasses, sugar beet molasses, potato peels and tomato peels. Each of these sources was replaced with glucose in the cultivation medium as control in a concentration of 100 g L^{-1} at initial pH of 6.0. Different parameters were determined and obtained results are listed in Table 1. As shown, great change in pH value was noticed up

Table 1: Effect of carbon source on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

			Single cell protein		Single cell oil	
Carbon source	Final pH value	Consumed sugar (g L^{-1})	Weight (g L^{-1})	Productivity dx/dt	Weight (g L^{-1})	Productivity dp/dt
Glucose (control)	5.3	36.2	3.6	0.050	1.2	0.017
Sugar cane molasses	4.9	30.3	2.5	0.035	1.6	0.022
Sugar beet molasses	7.2	16.6	3.2	0.044	1.8	0.025
Potato peels	4.6	18.4	2.2	0.031	1.6	0.022
Tomato peels	5.8	21.2	1.8	0.025	1.4	0.019

dx:Δ biomass, dt: Δ time, dp: Δ product

to the lowest value to be 4.6 which recorded with potato peels as carbon source. The highest pH value was observed with beet molasses being 7.2. For sugar consumed (g L^{-1}), the highest value found when glucose was used as carbon source to be 36.2 g L^{-1} while the lowest sugar consumed value was observed in case of sugar beet molasses using as carbon source to be 16.6 g L^{-1}. Results obtained by Syed *et al.* (2006) showed that glucose was the best carbon source between the five tested sources. Their results exhibited dry biomass production about 34.6 g L^{-1} and 5.8% of γ-linolenic acid from different strains belonging to Mucorales.

For the dry cell weight (g L^{-1}) obtained by the used yeast *Cryptococcus curvatus* NRRLY-1511, sugar beet molasses recorded the highest dry cell weight being 3.2 g L^{-1} as shown in Table 1. On the other hand, tomato peels showed the lowest value of obtained dry biomass to be 1.8 g L^{-1} compared to that value obtained with control being 3.6 g L^{-1}. The cell weight correlated with oil weight produced (g L^{-1}) since sugar beet molasses gave the highest produced single cell oil to be 1.8 g L^{-1}. This means that the increase fold equal to 0.89 and 1.5 for yeast cell weight and single cell oil weight compared to control, respectively. So, one can detect that sugar beet molasses showed to be the best agro-industrial by-product can be used in single cell oil production by *Cryptococcus curvatus* NRRLY-1511. Results of Syed *et al.* (2006) proved that tapioca starch was the best source for lipid production among four different carbon source namely sucrose, lactose, soluble starch and tapioca starch. Similar results have also been reported earlier (Somashekar *et al.*, 2002).

Certik *et al.* (1997) illustrated that carbohydrates are usually metabolized via the Embden-Myerhof Pathway to generate pyruvate or acetyl-CoA, which are then used for proteosynthesis, respiration and synthesis of other compounds including membrane and storage lipids. Furthermore, the efficiency of biomass and SCO are illustrated in Fig. 1.

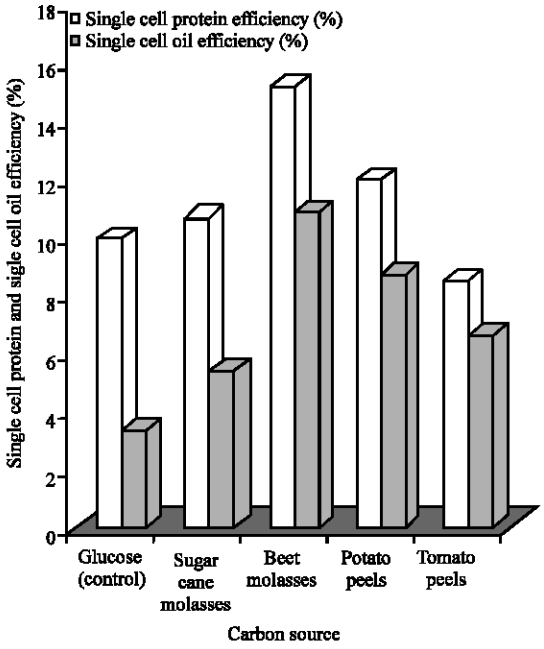

Fig. 1: Effect of carbon source on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

Carbon Source Concentration

In order to select the optimum concentration of the sugar beet molasses as carbon source, five concentrations were used as shown in Table 2. Tabulated data showed that the maximum sugar consumed was 36.2 g L^{-1} recorded in case of 175 g L^{-1} of sugar beet molasses. This result was not related to the dry cell weight since the growth of *Cryptococcus curvatus* NRRL Y-1511 was 2.3 g L^{-1} while the highest cell weight obtained was 4.6 g L^{-1} that obtained with 100 g L^{-1} of sugar beet molasses. The concentration of 125 g L^{-1} of carbon source was the optimum concentration required for single cell oil production being 1.2 g L^{-1} and oil percentage of 29.27%. This result was confirmed by that result obtained by Syed *et al.* (2006) who found that the increase in initial glucose leads to dry biomass decrease. This might be due to intolerance of the yeast cells to high concentration of glucose which increase the osmotic potential of the medium. In addition, the values of single cell protein (SCP) and SCO efficiencies are shown in Fig. 2.

Table 2: Effect of carbon source concentration on biomass and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

			Single cell protein		Single cell oil	
Carbon source concentration (g L^{-1})	Final pH value	Consumed sugar (g L^{-1})	Weight (g L^{-1})	Productivity dx/dt	Weight (g L^{-1})	Productivity dp/dt
75	5.3	25.2	3.5	0.049	0.75	0.010
100	5.2	28.1	4.6	0.064	0.92	0.013
125	5.4	26.6	4.1	0.057	1.20	0.017
150	4.8	32.4	2.5	0.035	0.85	0.012
175	5.0	36.2	2.3	0.032	0.89	0.012

dx: Δ Biomass, dt: Δ Time, dp: Δ Product

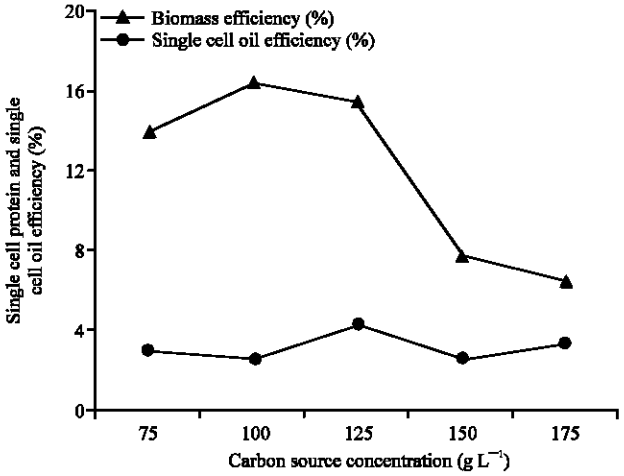

Fig. 2: Effect of carbon source concentration on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

Nitrogen Source

Four different of agro-industrial by-products were individually used as nitrogen sources with replaced each of them by $NaNO_3$, the nitrogen source in the cultivation medium as control. As shown in Table 3, little change in pH value of the cultivation medium was found up to the lowest value of 4.2 that recorded with rice bran as nitrogen source. For sugar consumptions, the highest value of sugar consumed was 65.37 g L^{-1} that recorded when corn gluten meal used as nitrogen source. The highest yield of dry biomass to be 2.8 g L^{-1} was also recorded with corn gluten meal that correlated with single cell oil weight

Table 3: Effect of nitrogen source on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

			Single cell protein		Single cell oil	
Nitrogen source	Final pH value	Consumed sugar (g L^{-1})	Weight (g L^{-1})	Productivity dx/dt	Weight (g L^{-1})	Productivity dp/dt
$NaNO_3$	5.5	39.35	3.5	0.049	1.2	0.017
Rice bran	4.2	26.56	1.3	0.018	1.0	0.014
Corn gluten	4.7	65.37	2.8	0.039	1.6	0.022
Corn steep liquor	5.3	23.95	2.5	0.035	1.05	0.015
Protelan	4.5	62.23	1.5	0.021	1.0	0.014

dx: Δ Biomass, dt: Δ Time, dp: Δ Product

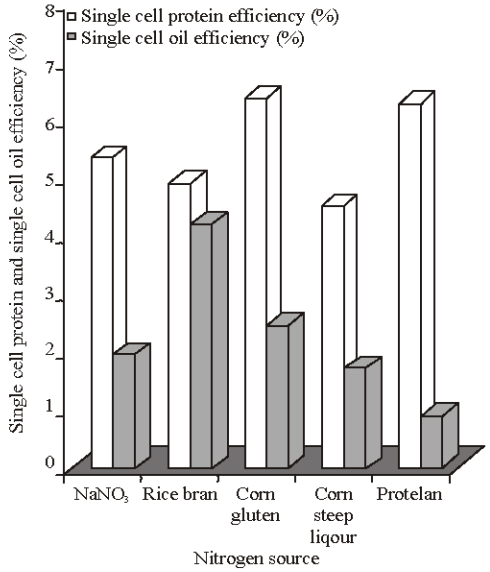

Fig. 3: Effect of nitrogen source on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

produced being 1.6 g L^{-1} and oil percentage of 64%. These results proved that corn gluten meal considered to be the most favourable nitrogen source required for single cell oil production by *Cryptococcus curvatus* NRRLY-1511. Furthermore, the efficiency of either SCP or SCO is illustrated in Fig. 3.

Nitrogen Source Concentration

Five concentrations of the nitrogen source were individually replaced in the cultivation medium to select the optimum concentration required for single cell oil production. Little change in final pH values was observed as shown in Table 4. *Cryptococcus curvatus*

Table 4: Effect of nitrogen source concentration on biomass and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

			Single cell protein		Single cell oil	
Nitrogen source concentration (g L^{-1})	Final pH value	Consumed sugar (g L^{-1})	Weight (g L^{-1})	Productivity dx/dt	Weight (g L^{-1})	Productivity dp/dt
0.13	5.1	36.60	3.2	0.044	1.5	0.021
0.195	5.2	24.40	3.2	0.044	1.2	0.017
0.26	4.9	34.45	3.1	0.043	1.1	0.015
0.325	5.2	35.00	2.9	0.040	1.0	0.014
0.39	5.4	32.00	2.1	0.029	1.1	0.015

dx: Δ Biomass, dt: Δ Time, dp: Δ Product

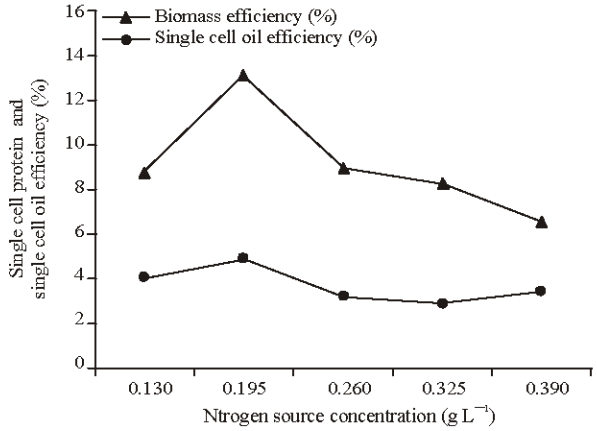

Fig. 4: Effect of nitrogen source concentration on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

NRRLY-1511 consumed 36.6 g L^{-1} sugars when using N-concentration in the cultivation medium 0.130 g L^{-1} that produced 3.2 g L^{-1} of dry biomass. The obtained SCO weight was equal to 1.5 g L^{-1} and oil percentage was 46.88%. Results obtained by Syed *et al.* (2006) showed that the total lipid content produced from the medium containing yeast extract was higher than that medium containing peptone. They reported that yeast extract was the best nitrogen source for obtaining biomass and lipid. In addition, the efficiency of either SCP or SCO is shown in Fig. 4.

Cultivation Medium pH

In order to examine the effect of initial pH value of the cultivation medium, seven levels of different pH were performed. Appreciate differences between the initial and final pH values were noticed as shown in Table 5. Little change in the final pH value was found between the different treatments. At pH 5.5 treatment, the dry cell weight of *Cryptococcus curvatus* NRRLY-1511 reached to 2.4 g L^{-1} with SCO weight of 1.7 g L^{-1}. On the other hand, pH 6.5 recorded the highest sugar consumption to be 56.2 g L^{-1}. It was found that microbial oil production was maximum when the mould was cultivated at pH 6.5 (Syed *et al.*, 2006). They also found that total lipid drastically decreased at pH 8.0 and at pH 4.0. They also reported that there was an increase in total lipid concentration in the pH range of 3.0 to 6.0. The efficiency of either SCP or SCO is illustrated in Fig. 5.

Table 5: Effect of initial pH value of the cultivation medium on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

			Single cell protein		Single cell oil	
Initial pH value	Final pH value	Consumed sugar (g L^{-1})	Weight (g L^{-1})	Productivity dx/dt	Weight (g L^{-1})	Productivity dp/dt
4.0	4.8	18.5	1.2	0.017	1.23	0.017
4.5	4.5	20.3	1.7	0.024	1.64	0.023
5.0	5.2	22.8	2.2	0.031	1.55	0.022
5.5	5.4	32.7	2.4	0.033	1.7	0.024
6.0	5.3	52.5	2.5	0.035	1.2	0.017
6.5	5.5	56.2	2.3	0.032	1.4	0.019
7.0	4.7	43.1	2	0.028	1.2	0.017

dx: Δ Biomass, dt: Δ Time, dp: Δ Product

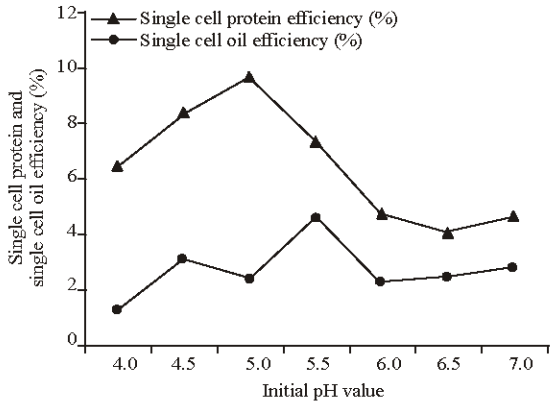

Fig. 5: Effect of initial pH value of the cultivation medium on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

Incubation Period

The behavior of *Cryptococcus curvatus* NRRL Y-1511 exhibited different activities with different incubation period as shown in Table 6. Very little change was found in final pH value of the cultivation medium. The highest value of sugar consumption was observed at 48 h to be 44.3 g L^{-1}. After 72 h of incubation, the dry biomass weight was 3.7 g L^{-1}. For microbial oil (SCO) weight production, data showed that 2.2 g L^{-1} of microbial oil with 59.46% oil percentage was produced after 72 h of incubation. This result was proved by plotting the efficiency of either SCP or SCO that illustrated in Fig. 6.

Table 6: Effect of incubation period on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

			Single cell protein		Single cell oil	
Incubation period (h)	Final pH value	Consumed sugar (g L^{-1})	Weight (g L^{-1})	Productivity dx/dt	Weight (g L^{-1})	Productivity dp/dt
24	5.2	26.2	1.6	0.022	1.0	0.014
48	5.1	44.3	2.4	0.033	1.4	0.019
72	5.2	28.4	3.7	0.051	2.2	0.030
96	5.3	32.6	3.2	0.044	2.2	0.031
120	5.6	30.8	3.4	0.047	2.2	0.031

dx: Δ Biomass, dt: Δ Time, dp: Δ Product

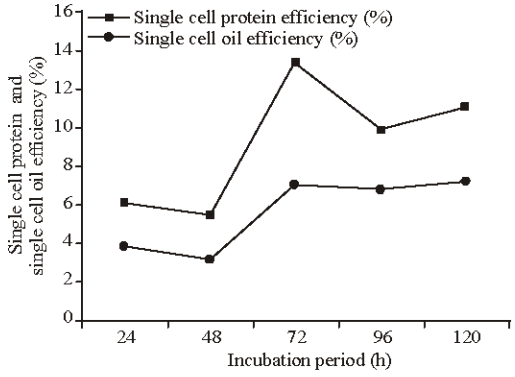

Fig. 6: Effect of incubation period on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

Growth Temperature

Six growth temperatures were used to examine their effect on the single cell oil production by *Cryptococcus curvatus* NRRLY-1511.As shown in Table 7, the treatment of 28°C exhibited change in final pH value of the cultural medium. In addition, the value of sugar consumed by the tested yeast strain that observed with the same treatment being 45.6 g L^{-1}. The highest production of either dry cell weight or SCO weight to be 5.0 g L^{-1}or 2.4 g L^{-1}, respectively. The growth of the tested yeast was low at temperature below 24°C and above 30°C as illustrated in Table 7. Carvalho *et al.* (1999) found that 28°C was the optimum temperature for dry biomass production. They obtained 2.47, 5.83 and 4.29 g L^{-1} dry biomass of *Mucor* sp. LB-54 at 12, 28 and 38°C, respectively. At the same temperature, the production of lipid content was 0.39, 1.21 and 0.49 g L^{-1}, respectively. Furthermore, the efficiency of either SCP or SCO is clearly shown in Fig. 7.

Table 7: Effect of growth temperature on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

			Single cell protein		Single cell oil	
Tested growth temperature (°C)	Final pH value	Consumed sugar (g L^{-1})	Weight (g L^{-1})	Productivity dx/dt	Weight (g L^{-1})	Productivity dp/dt
22	4.8	28.5	2.4	0.033	1.0	0.014
24	5.2	48.2	4.2	0.058	1.2	0.017
26	5.0	46.5	3.5	0.049	2.0	0.028
28	5.5	45.6	5.0	0.069	2.4	0.033
30	5.6	35.3	2.6	0.036	2.2	0.031
32	5.6	35.4	1.5	0.021	2.0	0.028

dx: Δ Biomass, dt: Δ Time, dp: Δ Product

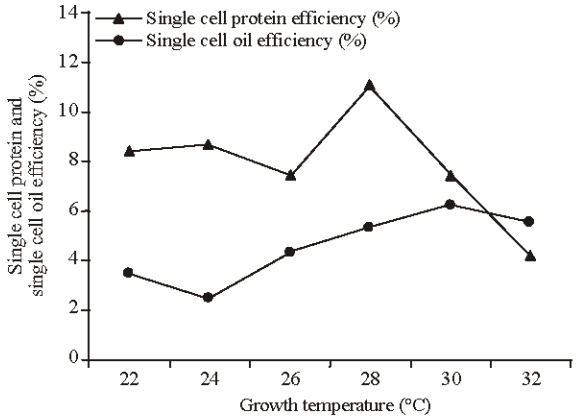

Fig. 7: Effect of growth temperature on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

Agitation Speed

The effect of agitation speed on the SCO production was investigated. Five agitation levels were performed and obtained results are listed in Table 8. From tabulated data one can see little change in the values of final pH of the cultural medium. Concerning the sugar consumption, it was very clear that *Cryptococcus curvatus* NRRLY-1511 used sugar in increase ratio with the increase agitation speed used. The increase of sugar consumption reached to the highest value to be 58.6 g L^{-1} in case of 200 rpm. The same trend was

observed with data of dry cell weight (SCP) since the values gradually increased to reach the highest value of 4.6 g L^{-1} that recorded in case of 200 rpm. Choi *et al.* (1982) reported that both biomass and its lipid content increased with increase in dissolved oxygen concentration. They added that *Rhodotorula glutinis* as obligate aerobic yeast, is dependent on oxygen for its energy metabolism and synthesis of cellular components. So, it is not surprising therefore, that increasing oxygen concentration showed a positive effect on the lipid content of these aerophilic yeast cells, even though lipid were more reduced than the other major components of living cells. Data of the efficiency of either SCP or SCO produced by the tested yeast strain is illustrated in Fig. 8.

Table 8: Effect of agitation speed of cultivation on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

			Single cell protein		Single cell oil	
Agitation speed (rpm)	Final pH value	Consumed sugar (g L^{-1})	Weight (g L^{-1})	Productivity dx/dt	Weight (g L^{-1})	Productivity dp/dt
100	5.1	26.2	1	0.014	0.5	0.007
125	5.3	28.4	1.3	0.018	0.8	0.011
150	5.4	34.2	2.2	0.031	1.2	0.017
175	4.4	52.4	3.4	0.047	1.4	0.019
200	4.2	58.6	4.6	0.064	2.2	0.031

dx: Δ Biomass, dt: Δ Time, dp: Δ Product

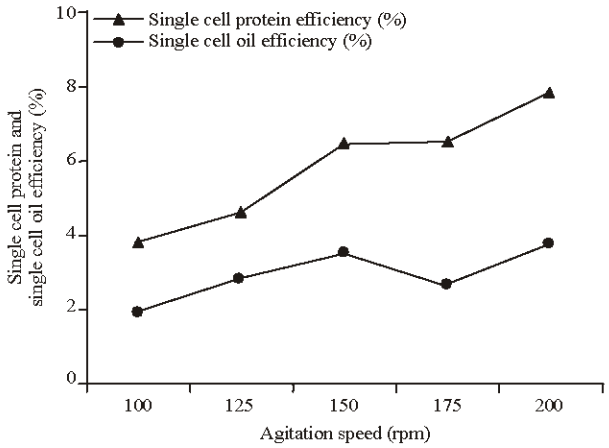

Fig. 8: Effect of agitation speed of cultivation on single cell protein and single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

Quantitative Values of Fatty Acids

Table 9 showing the profile of fatty acid composition of microbial oil (SCO) obtained by *Cryptococcus curvatus* NRRLY-1511 at 200 rpm agitation speed. This Table showing 15 appeared compounds for the tested sample and only nine detected for the control sample (stagnant culture). Of appeared compounds, 9 saturated fatty acids (n = 9) of 41.96%. The most incidence one was palmitic acid $C_{16}H_{32}O_2$ (C16:0) since it gave 16.74% of the tested sample. Of the total fatty acids, 26.91% was monosaturated fatty acids (n = 4). Oleic acid $C_{18}H_{34}O_2$ (C18:1 ω9) showed to be the highest one by 22.66% of the tested sample. The percent of polyunsaturated fatty acids (C18:2) was 31.13%. Of these, linoleic acid $C_{18}H_{30}O_2$ (C18:2 ω6) exhibited 30.68% of the tested sample.

Table 9: Quantitative values of fatty acids content in single cell oil production by an oleaginous yeast strain, *Cryptococcus curvatus* NRRL-Y-1511

Obtained fatty acids	Symbol	Tested sample	Control sample[a]	Canola oil[b] (Tower variety)
Caprylic	C8:0	1.554	-	-
Capric	C10:0	2.391	-	-
Lauric	C12:0	6.216	0.817	-
Myristic	C14:0	4.196	1.144	0.085
Myristoleic	C14:1	1.196	-	-
Pentadecanoic	C15:0	1.196	-	-
Cis-10-Pentadecanoic	C15:1	0.360	-	-
Palmitic	C16:0	16.736	12.873	0.839
Palmitoleic	C16:1	2.689	2.043	0.093
Heptadecanoic	C17:0	1.395	-	-
Stearic	C18:0	7.252	5.885	2.674
Oleic	C18:1	22.664	33.102	59.338
Linoleic	C18:2	30.683	42.297	23.578
Linolenic	C18:3	0.448	1.226	7.850
Arachidic	C20:0	0.797	0.613	3.151
Gadoleic	C20:1	-	-	1.636
Behenic	C22:0	-	-	0.033
Erucic	C22:1	-	-	0.785
Total saturated	(%)	41.960	21.332	6.782
Monounsaturated	(%)	26.909	35.145	61.852
Polyunsaturated	(%)	31.131	43.523	31.428
Total unsaturated	(%)	58.040	78.668	93.280

[a]SCO obtained from stagnant cultivation. [b]After Abou El-Hawa *et al.* (2004)

The importance of obtained microbial oil that is near close to canola oil in its composition. So, comparing the obtained microbial oil with canola oil it can be found, more or less, the same fatty acids with different concentration; Palmitic acid (16:0), Oleic (18:1), Linoleic (18:2), Linolenic (18:3), and Myristic (14:0) as reported by Abou El-Hawa *et al.* (2004).

CONCLUSIONS

With this strategy, it is able to stimulate single cell oil (SCO) production by *Cryptococcus curvatus* NRRLY-1511 from the cultivation medium containing some agro-industrial by products as carbon and nitrogen sources. The data presented in this investigation showed the significant influence of some nutritional and environmental factors. So these results suggest that *Cryptococcus curvatus* NRRLY-1511 may have potential for commercial development for the production of single cell oil (SCO) by fermentation technique.

REFERENCES

Abou El-Hawa, S.H., W.S. Ragab, R.A. El-Dengawy and F.F. Ali, 2004. Composition of Canola seed oil. Yemeni J. Sci., 6: 25-35.

Carvalho, P.O., J.G. Oliveira and G.M. Pastore, 1999. Enhancement of gamma-linolenic acid production by the fungus *Mucor* sp. LB-54 by growth temperature. Rev. Microbiol., 30: 170-175.

Certik, M., L. Balteszova and J. Sajbidor, 1997. Lipid formation and gamma-linolenic acid production by Mucorales fungi grown on sunflower oil. Lett. Applied Microbiol., 25: 101-105.

Choi, S.Y., D.Y. Ryu Dewey and J.S. Rhee, 1982. Production of microbial lipid: Effects of growth rate and oxygen on lipid synthesis and fatty acid composition of *Rhodotorula gracilis*. Biotechnol. Bioeng., 24: 1165-1172.

Granger, L.M., P. Perlot, G. Goma and A. Pareilleux, 1992. Kinetics of growth and fatty acid production of *Rhodotorula glutinis*. Applied Microbiol. Biotechnol., 37: 13-17.

Granger, L.M., P. Perlot, G. Goma and A. Pareilleux, 1993. Effect of various nutrient limitations on fatty acid production by *Rhodotorula glutinis*. Applied Microbiol. Biotechnol., 38: 784-789.

Hansen, L.U. and M.C.M. Chiu, 2005. Isolation and composition of chromoplasts from tomatoes. J. Agric. Food Chem., 53: 6678-6682.

Herbert, D., P.J. Phipps and R.E. Strange, 1971. Chemical Analysis of Microbial Cells. In: Methods in Microbiology, Norris, J.R. and D.W. Ribbons (Eds.). Vol. 5, Academic Press, London, New York, pp: 209 -344.

Lindberg, A.M. and G. Molin, 1993. Effect of temperature and glucose supply on the production of polyunsaturated fatty acids by the fungus *Mortierella alpina* CB S343.66 in fermentor cultures. Applied Microbiol. Biotechnol., 39: 450-455.

Pan, L.X., D.F. Yang, L. Shao, W. Li, G.G. Chen and Z.Q. Liang, 2009. Isolation of the oleaginous yeasts from the soil and studies of their lipid-producing capacities. Food Technol. Biotechnol., 47: 215-220.

Pandey, K. and P.K. Agarwal, 1993. Effect of EDTA, potassium ferrocyanide, and sodium potassium tartrate on the production of ethanol from molasses by *S. cerevisiae*. Enzy. Microbial Technol., 15: 887-898.

Radwan, S., 1978. Coupling of two dimensional thin layer chromatography with gas liquid Chromatography for the quantitative analysis of lipid classes and their constituent fatty acids. J. Chromat. Sci., 16: 538-542.

Renaud, S., 1990. Linoleic acid, platelet aggregation and myocardial infarction. Atherosclerosis, 80: 255-256.

Somashekar, D., G. Venkateshwaran, K. Sambaiah and B.R. Lokesh, 2002. Effect of culture conditions on lipid and gamma-linolenic acid production by mucoraceous fungi. Process Biochem., 38: 1719-1724.

Sorger, D., K. Athenstaedt, C. Hrastnik and G. Daum, 2004. A yeast strain lacking lipid particles bears a defect in ergosterol formation. J. Biol. Chem., 279: 31190-31196.

Syed, M.A., S.K. Singh, A. Pandey, S. Kanjilal and R.B.N. Prasad, 2006. Effects of various process parameters on the production of ã-Linolenic acid in submerged fermentation. Food Technol. Biotechnol., 44: 283-287.

Zweytick, D., K. Athenstaedt and G. Daum, 2000. Intracellular lipid particles of eukaryotic cells. Biochim. Biophys. Acta., 1469: 101-120.