

## CONVERSION OF CORN PROTELAN TO MICROBIAL OIL BY *Rhodotorula glutinis*

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### ABSTRACT

Protelan is a waste of starch and glucose factory. *Rhodotorula glutinis* ARC-y-54 strain which capable of oil biosynthesis on a medium containing agri-industrial wastes such as sugar cane molasses as a sole carbon source and protelan as a sole nitrogen source. The optimization for single cell oil (SCO) production was carried out and both biomass and SCO production were determined. Obtained data showed that the optimum conditions for SCO production were 48 h, 20 °C, 5.5, 200 rpm for incubation period, incubation temperature, initial pH and agitation speed, respectively.

The effect of NaCl addition was also examined. Obtained data proved the possibility of using *Rhodotorula glutinis* ARC-y-54 for single cell oil (SCO) production using some agri-industrial by-products from economic point of view and for environmental protection as well.

### INTRODUCTION

The world wide impending demand for oils has led to a search for unconventional sources. Microbial production of oil appears to be an attractive solution particularly in those countries with restricted supplies of oil for natural sources (Moreton, 1985). *Rhodotorula glutinis* ARC-y-54 is one of the principal oleaginous yeasts used for oil production being non-toxic and relatively easy to grow and harvest as reported by Misra *et al.*, (1984). The optimization for oil production by the tested yeast *Rhodotorula glutinis* ARC-y-54 was examined in this investigation.

### MATERIALS AND METHODS

#### Materials :

#### Yeast strain :

The yeast strain used in this investigation was *Rhodotorula glutinis* ARC-y-54. This strain was kindly provided from Prof. D r. Samir EL-Sayed, Prof. fermentation Technol., ARC, Giza, Egypt.

#### Cultivation medium :

The cultivation medium of Anne and Molin (1993) was used for cultivation of *Rhodotorula glutinis* ARC-y-54. The yeast extract malt broth medium (NRRL) was used for inoculum preparations and maintenance of the yeast strain as well.

**Wastes used :**

Sugar cane molasses (SCM) that obtained from sugar cane factory of Belkas, Dakahlia Governorate was used as a sole carbon source. This by-product containing 48 % total sugar before purification that became 23.3 % after purification. Protelan obtained from starch and glucose factory was used as a sole nitrogen source. This waste containing 0.40 % nitrogen. Each of these wastes was replaced with the other source in the cultivation medium used in the same ratio.

**Methods :**

**Preparation of sugar cane molasses :**

The sample of sugar cane molasses (SCM) was prepared by diluting with water in an equal volume using the method of Pundey and Agarwal, (1993) with little modification. H<sub>2</sub>SO<sub>4</sub> solution was used to reduce the pH value to reach 3.0. Sample was boiled at 100 °C for 1 hr. then maintained at room temperature for 24 hr., centrifuged and filtered. Filtrated solution was used to determine the total sugar to be used as a sole carbon source.

**Preparation of nitrogenous wastes :**

Nitrogenous waste namely protelan, was oven dried at 105 °C for 2 hr. and milled. About 7.5 g of waste was added to 100 ml of 1.5 % H<sub>2</sub>SO<sub>4</sub> and autoclaved for 45 minutes then filtered. The obtained supernatant was attained at pH 6, being used as a sole nitrogen source (Dokhan, 2005).

**Microbiological procedures :**

Six carbon sources namely glucose syrup, beet molasses, sugar cane molasses, potatoes peel, tomatoes peel and squash peel were added to the basal medium at a concentration of 10 % (W/V) to study the effect of each carbon source on production of microbial oil. The experiments were carried out at 30 °C for different times.

Rhodotorula strain was grown on the production medium provided with sugar cane molasses as a sole carbon source. Different concentrations of sugar cane molasse ranging from 80 g/L<sup>-1</sup> to 180 g/L (W/V) were used to select the optimal concentration.

**Preparation of the inoculum :**

The inoculum used in the experiments was prepared in Erlenmeyer flasks on YM broth (NRRL) medium. The cultivation conditions were carried out at 30 °C; pH 6; 48 hr; and shake speed was 150 rpm/min. The inoculum size of 3 % v/v was added to the test medium.

**Fermentation process with free yeast cells :**

The previously mentioned fermentation medium was put in 250 ml capacity Erlenmeyer flasks, each flask received 100 ml of the prepared medium, and initial pH was adjusted to 5.5 using a pH meter before autoclaving at 121 °C for 20 min. then inoculated with 3 % v/v of cells suspension of the examined strain in sterile distilled water. The cultures were incubated at the experimental temperatures on a rotary shaker at 150 rpm for the required fermentation periods using LAB-line instrument, Inc., plaza, Mel Rose, Park, ILL. 60160.

**Yeast dry weight determination :**

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

$$\text{Growth yield efficient} = \frac{\text{Cell dry weight gL}^{-1}}{\text{Sugar consumed gL}^{-1}} \times 100$$

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

$$\text{Microbial oil productivity} = \frac{\text{Microbial oil weight gL}^{-1}}{\text{Cell dry weight gL}^{-1}} \times 100$$

**Determination of total sugars :**

Total sugars were determined according to the method of Herbert *et al.*, (1971) as follow : In to thick walled tubes, 1 ml of tested sample was pipetted and well mixed with 1 ml of 5 % phenol solution, then 5 ml of concentrated sulphuric acid were directly added on the surface of liquid with shaking. The tubes were allowed to stand in water bath at 25 °C from 10 to 20 min. before reading the density of obtained colour at 490 nm. using a spectrophotometer. The standard was carried out using glucose. The total sugars was expressed as mg/ml according to the equation of  $y = 0.0274x + 0.024$  with  $r^2 = 0.9586$ .

**Microbial oil extraction :**

Microbial oil extraction was carried out according to the method of Granger *et al.* (1993) with little modification 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 for 72 hr. to constant weight. The dried cells were then milled for 20 min. with carbonium powder and extracted in a condenser unit at 60-70 °C, then filtered using a filter paper watman No.1 and oven dried at 70 °C. The percentage of fatty acids composition in triglycerides efficiency of oil biosynthesis was calculated per unit of medium volume or per 100 g of sugars utilized by the yeast. The oil yield efficient was also calculated according to the following equation :

$$\text{Microbial oil yield efficiency} = \frac{\text{Microbial oil weight gL}^{-1}}{\text{Sugar consumed gL}^{-1}} \times 100$$

**Fatty acids measurement :**

The method of extraction for measurement of fatty acids of obtained microbial oil was carried out according to the method of Radwan (1978). Twenty five mg sample + 2.5 ml methanolic sulfuric acid (1 ml conc. H<sub>2</sub>SO<sub>4</sub> + 100 ml methanol) + 1 ml benzene were put in a well closed tube. The tube

was inserted in water bath at 90 °C or in oven at 90 °C for 90 min. The tube was allowed to cool then 4 ml distilled H<sub>2</sub>O was added + 2.5 ml petroleum ether and shaken well. The ether layer (upper layer) was removed in a small vial and evaporated. Then at injection 50 ml n. hexane was added. The tested sample was injected in HP (Hewlett Packard) 6890 GC instrument with: Detector : FID (Flameionization detector) and Detector temperature : 250 °C.

## RESULTS AND DISCUSSION

### Selection of carbon source :

Six of agri-industrial by-products used as carbon sources were individually examined for microbial oil production by the tested yeast *Rhodotorula glutinis* ARC-y-54 namely glucose syrup, sugar cane molasses, beet molasses, potato, squash and tomatoes peel. Each of these sources was replaced with glucose in the cultivation medium as control in a concentration of 100 gL<sup>-1</sup> at initial pH of 6.0. Different parameters were determined and obtained results are listed in Table 1. As shown in the Table, great change in pH value up to the lowest value to be 1.5 which recorded with glucose syrup as carbon source. The highest pH values observed with tomatoes peel being 8.35. For sugar consumed (gL<sup>-1</sup>), the highest consumed sugar was found when sugar cane molasses was used as carbon sources to be 52.6 gL<sup>-1</sup> while the lowest sugar consumed was observed in case of squash peel using as carbon source comparing to the control value of 36.8 gL<sup>-1</sup> when using glucose as carbon source. Results obtained by Syed *et al.* (2006) showed that glucose was the best carbon source between the four tested sources. Their results exhibited dry biomass production about 34.6gL<sup>-1</sup> and 5.8 % of  $\gamma$ -linolenic acid from different strains belonging to Mucorales.

**Table 1: Effect of agri-industrial by-products as carbon sources on oil and biomass production.**

Carbon source	Final pH	Sugar consumed g/L	Cell weight gL <sup>-1</sup>	Oil weight gL <sup>-1</sup>	Oil percentage %	Oil yield efficient %	dx/dt	dp/dt	Growth yield efficient
Glucose syrup	1.50	40.21	5.117	1.14	22.252	2.83	0.1066	0.0237	12.72
Sugar cane molasses	4.53	52.6	5.951	1.45	24.361	2.75	0.1239	0.0302	11.313
Beet molasses	5.41	46.8	3.000	0.65	21.66	1.38	0.0625	0.0135	6.410
Potatoes peel	3.87	43.16	3.758	0.75	19.95	4.05	0.0678	0.00135	7.548
Squash peel	6.37	36.8	3.852	0.652	16.92	1.77	0.080	0.0135	10.467
Tomatoes peel	8.35	37.2	3.12	0.422	13.5541	2.52	0.1443	0.0195	8.387
Glucose (control)	4.63	35.4	5.14	0.933	18.15	2.63	0.1070	0.0194	14.51

dx/dt cell productivity, gm dry weight/L/hr  
dp/dt oil productivity, gm oil/L/hr

For the cell weight ( $\text{gL}^{-1}$ ) obtained of the used yeast *Rhodotorula glutinis* ARC-y-54, sugar cane molasses (SCM) recorded the highest cell weight being  $5.951 \text{ gL}^{-1}$  (Table 1). On the other hand, beet molasses showed the lowest value of obtained biomass to be  $3.000 \text{ gL}^{-1}$  compared to that value obtained with control being  $5.14 \text{ gL}^{-1}$ . The cell weight correlated with oil weight produced  $\text{gL}^{-1}$  since sugar cane molasses gave the highest produced oil to be  $1.45 \text{ gL}^{-1}$ . This means that the increase fold equal to 1.15 and 1.55 for cell weight and oil weight compared to control, respectively. So one can detect that sugar cane molasses showed to be the best by-product can used in oil production by *Rhodotorula glutinis* ARC-y-54. Results of Syed *et al.* (2006) proved that glucose was the best carbon source with dry biomass production of  $34.6 \text{ gL}^{-1}$ . They also found 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, 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.

**Concentration of carbon source :**

In order to select the optimum concentration of the sugar cane molasses, six concentrations were used as shown in Table 2. Tabulated data showed that the maximum sugar consumed was  $67.3 \text{ gL}^{-1}$  recorded in case of  $160 \text{ gL}^{-1}$ . This result was not related to the cell weight since the growth of *Rhodotorula glutinis* ARC-y-54 was  $5.68 \text{ gL}^{-1}$ . This means that the concentration of  $160 \text{ gL}^{-1}$  of carbon was the optimum concentration required for microbial oil production being  $2.01 \text{ gL}^{-1}$ . Data showed also that oil decreased when using  $180 \text{ gL}^{-1}$  which might be due to intolerance of the cells to high concentration of glucose in used source that the increase of the osmotic potential of the medium. This was clear in low sugar cane concentration that not so effective in oil production giving  $0.84 \text{ gL}^{-1}$  with  $80 \text{ gL}^{-1}$ .

**Table 2: Effect of sugar cane molasses concentration on microbial oil and biomass production.**

C-source concent., $\text{gL}^{-1}$	Finial pH	Sugar consumed $\text{g/L}$	Cell weight $\text{gL}^{-1}$	Oil weight $\text{gL}^{-1}$	Oil percentage %	Oil yield efficient %	dx/dt	dp/dt	Growth yield efficient%
80	5.20	39.81	3.012	0.84	27.88	2.11	0.0627	0.0175	7.565
100	4.90	44.51	4.076	1.25	30.712	2.80	0.0847	0.0260	9.144
120	5.00	47.28	4.52	1.44	31.858	3.045	0.0941	0.03	9.560
140	5.5	58.6	5.85	2.03	34.522	3.46	0.1218	0.0422	9.982
160	5.30	67.3	5.68	2.01	35.38	2.98	0.1183	0.0418	8.439
180	5.10	60.15	5.85	1.89	32.30	3.14	0.1218	0.0393	9.725

dx/dt cell productivity, gm dry weight/L/hr

dp/dt oil productivity, gm oil/L/hr

**Selection of nitrogen source :**

Four different agri-industrial by-products were used as nitrogen sources with replaced each of them by the N-source in the cultivation medium  $\text{NH}_4\text{Cl}$  as control. As shown in Table 3, little change in pH value of the

cultivation medium was found up to the lowest values of 4.76 that recorded with protelan as N-source. For sugar consumptions, the highest sugar consumed was 66.5 gL<sup>-1</sup> that recorded when protelan used as N-source. The highest yield of biomass to be 4.22 gL<sup>-1</sup> was also recorded with protelan that correlated with microbial oil weight produced being 1.49 gL<sup>-1</sup>. These results proved that protelan considered to be the most favourable N-source required for microbial oil production by *Rhodotorula glutinis* ARC-y-54. The use of protelan as N-source may help to reduce the cost of producing the microbial oil by such oleaginous yeast strain.

**Table 3. Effect of agri-industrial by-products as N source on microbial oil and biomass production.**

Nitrogen source	Final pH	Sugar consumed g/L	Cell weight gL <sup>-1</sup>	Oil weight gL <sup>-1</sup>	Oil percentage %	Oil yield efficient %	dx/dt	dp/dt	Growth yield efficient %
Corn gluten	5.02	55.54	3.85	1.22	31.688	2.196	0.080	0.025	6.931
Corn steep Liquer	5.60	1.59	3.175	1.03	32.440	2.136	0.066	0.0214	6.5871
Protelan	4.76	66.5	4.22	1.49	36.07	2.24	0.0879	0.0310	6.34
Rice bran	4.85	1.66	1.100	0.335	30.45	0.66	0.0229	0.0069	2.1825
NH <sub>4</sub> Cl (Control)	4.90	44.51	4.076	1.25	30.712	2.80	0.0874	0.0260	9.144

dx/dt cell productivity, gm dry weight/L/hr

dp/dt oil productivity, gm oil/L/hr

**Concentration of nitrogen source :**

Nine concentrations of the selected N-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. *Rhodotorula glutinis* ARC-y-54 consumed 75.0 gL<sup>-1</sup> sugar when using N-concentration in the cultivation medium 0.065 gL<sup>-1</sup> that produced 6.50 gL<sup>-1</sup> of biomass. The obtained oil weight was equal to 2.45 gL<sup>-1</sup>. Results obtained by Syed et al. (2006) showed that total lipid content produced from the medium containing yeast extract was higher than that medium containing peptone.

**Table 4: Effect of N-source concentration on microbial oil and biomass production.**

N-source concent., gL <sup>-1</sup>	Final pH	Sugar consumed g/L	Cell weight gL <sup>-1</sup>	Oil weight gL <sup>-1</sup>	Oil percentage %	Oil yield efficient %	dx/dt	dp/dt	Growth yield efficient%
0.032	4.87	50.2	4.80	1.52	31.66	3.02	0.1	0.0316	9.56
0.065	4.94	75.0	6.50	2.45	37.69	3.26	0.1354	0.0510	8.66
0.130	4.92	72.4	5.23	1.87	35.75	2.58	0.1089	0.038	7.223
0.195	5.13	71.5	4.55	1.45	31.86	2.02	0.094	0.030	6.363
0.260	4.6	69.5	3.90	1.20	30.76	1.72	0.0812	0.025	5.611
0.320	5.15	66.7	4.00	1.21	31.43	1.814	0.0822	0.025	5.99
0.390	5.50	63.7	4.20	1.15	28.98	1.80	0.0875	0.023	6.593
0.455	5.19	56.2	4.20	1.03	24.00	1.82	0.0875	0.0214	7.460
0.520	4.84	49.5	4.01	1.00	24.00	2.02	0.0835	0.0208	8.101

dx/dt cell productivity, gm dry weight/L/hr

dp/dt oil productivity, gm oil/L/hr

They reported that yeast extract was the best nitrogen source for obtaining biomass and lipid.

**Effect of cultivation medium pH :**

In order to examine the effect of initial pH value of the cultivation medium, seven values of different pH were performed. Appreciate differences between the initial and final pH values were noticed compared to control pH 6 as shown in Table 5. Little change in the final pH values was found between the different treatments. At pH 5.0 treatment, the cell weight of *Rhodotorula glutinis* ARC-y-54 reached to 2.36 gL<sup>-1</sup> with oil weight of 0.886 gL<sup>-1</sup>. When using initial pH of 5.5, 4.70 gL<sup>-1</sup> of *Rhodotorula glutinis* ARC-y-54 was obtained with 1.98 gL<sup>-1</sup> of single cell oil. On the other hand, pH 6 recorded high sugar consumption to be 112.11 gL<sup>-1</sup> and high cell weight 4.30 gL<sup>-1</sup>. Wagner and Daum (2005) found that the pH tends to drop in the early stages of a growth of *Rhodotorula glutinis* ARC-y-54. They also stated that fluctuation in pH did not appear to be correlated with the presence or absence of available sugar in the growth medium

**Table 5: Effect of initial pH of cultivation medium on microbial oil and biomass production.**

Initial pH	Final pH	Sugar consumed g/L	Cell weight gL <sup>-1</sup>	Oil weight gL <sup>-1</sup>	Oil percentage %	Oil yield efficient %	dx/dt	dp/dt	Growth yield efficient%
4	4.5	32.5	1.411	0.219	15.520	0.673	0.0293	0.0045	4.3415
4.5	4.16	42.4	2.65	0.75	28.30	1.76	0.0552	0.0156	6.25
5	5.21	75.5	2.36	0.886	37.54	1.17	0.049	0.0184	3.1258
5.5	5.17	117.5	4.70	1.98	42.127	1.68	0.097	0.04125	4.00
6	5.4	112.11	4.30	1.75	40.697	1.56	0.089	0.036	3.835
6.5	5.34	104.2	3.50	1.33	38.00	1.276	0.0729	0.0277	3.358
7	4.18	87.3	1.101	0.14	12.715	0.160	0.0229	0.0029	1.261

dx/dt cell productivity, gm dry weight/L/hr

dp/dt oil productivity, gm oil/L/hr

**Effect of NaCl :**

The effect of NaCl on the yeast oil production was examined using six different concentrations of NaCl. The final pH value of the cultural medium changed to the lowest value to be 3.5 that recorded with 15 % NaCl compared to control without NaCl. Negative correlation between NaCl concentration and sugar consumption was found. The consumed sugar decreased gradually to the lowest value being 26.1 gL<sup>-1</sup> that recorded with 15 % NaCl. For cell weight of *Rhodotorula glutinis* ARC-y-54, the obtained weight was gradually decreased with increase of NaCl to the lowest value up to 0.200 gL<sup>-1</sup> with 15 % NaCl. The same trend of negative correlation was also found with oil production since the lowest value of microbial oil obtained was 0.028 gL<sup>-1</sup> that found in case of 15 % NaCl as shown in Table 6. Andreishcheva *et al.* (1999) stated that NaCl concentration significantly affect the final biomass yield and consequently the lipid production. They also added that the pool of free fatty acids rose, most likely due to their less active utilization for the synthesis of triacylglycerols under salt stress.

**Table 6: Effect of NaCl addition on the microbial oil and biomass production.**

NaCl concentration (%)	Final pH	Sugar consumed g/L	Cell weight gL <sup>-1</sup>	Oil weight gL <sup>-1</sup>	Oil percentage %	Oil yield efficient %	dp/dt	dx/dt	Growth yield efficient%
1	4.1	38.8	3.227	1.30	40	3.350	0.0672	0.0270	8.317
3	4.0	31.5	3.948	1.50	38.07	3.940	0.08225	0.03125	12.53
5	4.6	33.14	1.139	0.40	35.11	1.207	0.0237	0.0083	3.4369
7	4.3	32.4	2.722	0.70	25.92	2.16	0.0567	0.0145	8.401
10	5.0	27.5	0.210	0.029	13.8	0.105	0.004	0.00060	0.763
15	3.5	26.1	0.200	0.028	14.0	0.107	0.004	0.0005	0.766
Control	5.3	87.4	5.55	2.30	41.44	3.63	0.1156	0.0625	6.350

dx/dt cell productivity, gm dry weight/L/hr

dp/dt oil productivity, gm oil/L/hr

**Growth temperature :**

Six growth temperature were used to examine their effect on the single cell oil production by *Rhodotorula glutinis* ARC-y-54. As shown in Table 7, the treatment of 20 °C exhibited very little change in final pH value of the cultural medium. In addition, the highest sugar consumed by the tested yeast strain was observed with the same treatment being 115.5 gL<sup>-1</sup>. This was correlated with the highest production of either cell weight or oil weight to be 5.94 gL<sup>-1</sup> or 2.44 gL<sup>-1</sup> for cell weight or oil weight, respectively. Syed *et al.* (2006) found that the lipid content increase when the mould was cultivated at 20 °C more than mould was cultivated at 35 °C. They also found that the degree of unsaturation was higher in the microorganisms cultivated at lower temperature than in microorganisms cultivated at higher temperature. Similar results have also been reported by Hiruta *et al.*, (1996).

**Table 7: Effect of growth temperature on microbial oil and biomass production.**

Temp. °C	Final pH	Sugar consumed g/L	Cell weight gL <sup>-1</sup>	Oil weight gL <sup>-1</sup>	Oil percentage %	Oil yield efficient %	dx/dt	dp/dt	Growth yield efficient
15	5.4	85.2	5.12	1.81	35.35	2.12	0.11	0.04	6.00
20	5.6	115.5	5.94	2.44	41.07	2.112	0.12	0.05	5.14
25	5.2	89.4	5.23	2.11	40.34	2.360	0.11	0.04	5.85
30	5.6	97.3	5.51	2.20	39.90	2.261	0.11	0.05	5.66
35	5.3	78.3	6.31	2.19	34.70	2.796	0.13	0.05	8.05
40	5.01	31.12	2.41	0.43	17.842	1.38	0.05	0.01	7.74

dx/dt cell productivity, gm dry weight/L/hr

dp/dt oil productivity, gm oil/L/hr

**Incubation period :**

The behaviour of *Rhodotorula glutinis* ARC-y-54 exhibited different activities with different incubation period as shown in Table 8. 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 87.4 gL<sup>-1</sup>. After 48 h

incubation the biomass weight was 5.55 gL<sup>-1</sup>. For oil weight production, data showed that 2.30 gL<sup>-1</sup> of single cell oil was produced after 48 hr of incubation at 20 °C.

**Table 8: Effect of incubation period on microbial oil and biomass production.**

Incubation period, hr	Final pH	Sugar consumed g/L	Cell weight gL <sup>-1</sup>	Oil weight gL <sup>-1</sup>	Oil percentage %	Oil yield efficient %	dx/dt	dp/dt	Growth yield efficient %
24	5.08	55.4	3.19	1.02	31.974	1.841	0.0664	0.02125	5.758
48	5.3	87.4	5.55	2.30	41.44	2.63	0.1156	0.0479	6.350
72	5.10	85.5	5.00	2.00	40.00	2.33	0.1041	0.0416	5.84
96	5.3	82.6	5.67	2.21	38.97	2.67	0.1181	0.0460	6.86
120	5.4	74.1	5.32	1.85	34.77	2.49	0.110	0.038	7.179

dx/dt cell productivity, gm dry weight/L/hr

dp/dt oil productivity, gm oil/L/hr

**Agitation effect :**

The effect of agitation on the single cell oil production was investigated. Five agitation levels were performed and obtained results are listed in Table 9. 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 *Rhodotorula glutinis* ARC-y-54 used sugar in increase ratio with the increase of agitation used. The increase of sugar consumption reached to the highest value to be 130.0 gL<sup>-1</sup> in case of 200 rpm. The same trend was observed with data of cell weight since the values increased gradually to reach the highest values of 6.188 gL<sup>-1</sup> that recorded in case of 200 rpm. Again data of bio-oil produced by the tested yeast showed also gradual increase up to 2.75 gL<sup>-1</sup> with 200 rpm, too. Wagner and Daum (2005) reported that aeration of yeast in a phosphate-molasses medium resulted in a rapid consumption of sugar during the whole period of aeration. This was accompanied by an increase in the weight of yeast (*Rhodotorula glutinis* ARC-y-54), accounted for by an increase in all constituents, fat, carbohydrate, mineral matter and a small amount of protein formed from the residual nitrogen carried over from the growth medium.

**Table 9: Effect of agitation speed of cultivation on microbial oil and biomass production.**

Agitation speed, rpm	Final pH	Sugar consumed g/L	Cell weight gL <sup>-1</sup>	Oil weight gL <sup>-1</sup>	Oil percentage %	Oil yield efficient %	dp/dt	dx/dt	Growth yield efficient%
100	5.5	44.5	2.300	0.688	29.91	1.546	0.0479	0.014	5.168
125	5.1	52.4	3.00	0.949	31.63	1.811	0.0625	0.019	5.72
150	5.20	78.16	3.783	1.55	40.97	1.983	0.0788	0.0322	4.840
175	4.8	90.71	3.890	1.65	42.41	1.818	0.0810	0.0343	4.288
200	4.7	130.2	6.188	2.75	44.4	2.112	0.0573	0.1289	4.752

dx/dt cell productivity, gm dry weight/L/hr

dp/dt oil productivity, gm oil/L/hr

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تحويل بروتيلان الذرة إلى زيت ميكروبي باستخدام خميرة الرودوتوريولا جلوتينيز  
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بروتيلان الذرة هو مخلف لمصنع النشا والجلوكوز وخميرة الرودوتوريولا من  
الخمائر القادرة على تصنيع الزيت الميكروبي على بيئة تحتوى على مخلف زراعي صناعي  
مثل مولاس قصب السكر وبروتيلان الذرة كمصدر وحيد للنتروجين. وقد تم دراسة  
الظروف المثلى لإنتاج الزيت الميكروبي والكتلة الحيوية وبينت النتائج المتحصل عليها أن  
أنسب الظروف لإنتاج الزيت الميكروبي هي 48 ساعة ، 20 °م ، 5.5 ، 200 لفة وذلك  
لكل من فترة التحضين ، درجة الحرارة للنمو الميكروبي النامي ببيئة الزراعة وكذلك درجة  
الـ pH وعدد لفات سرعة الهز المستخدمة في التحضين. كذلك تم دراسة تأثير إضافة  
كلوريد الصوديوم على إنتاج الزيت الميكروبي. ولقد أثبتت النتائج المتحصل عليها إمكانية  
إستخدام سلالة الخميرة تحت الدراسة *Rhodotorula glutinis* ARC-y-54 لإنتاج  
الزيت الميكروبي بإستخدام بعض المخلفات الزراعية الصناعية وذلك من وجهة النظر  
الإقتصادية وكذلك لحماية البيئة.