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PRODUCTION OF SINGLE CELL OIL FROM CORN GLUTEN MEAL BY *Candida lipolytica*

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ABSTRACT

Corn gluten meal is a waste of starch and glucose factory. *Candida lipolytica* NRRL-y-1095 strain which capable of oil biosynthesis on a medium containing Agro-industrial wastes such as sugar cane molasses and corn gluten meal. The optimization for single cell oil (SCO) production was carried out and both biomass and SCO produced were determined. Obtained data showed that the optimum conditions for SCO production were 72 h, 20 ºC, 6, 200 rpm for incubation period, incubation temperature, initial pH and agitation speed, respectively. The effect of NaCl addition was also examined and the obtained data proved the possibility of using *Candida lipolytica* NRRL-y-1095 for single cell oil (SCO) production using some agri-industrial by-products from economic point of view and for environmental protection as well.

INTRODUCTION

One of the possibilities of by-products processing obtained from the food industry is to utilize them for the cultivation of microorganisms in order to produce biomass. The efficiency of oil 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. The main storage lipids in eukaryotes are triacyl-glycerol. Storage lipids are usually found within special organelles known 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).

In this work an attempt was made to biosynthesize oil by cultivating selected yeast strains on sugar cane molasse with corn gluten containing medium. The aim of this study was to determine the optimal conditions for oil biosynthesis by *Candida lipolytica* NRRL-y-1095. So as to increase the utilization of sugar cane molasse and corn gluten meal for the production of oil-protein preparations for many purposes such as poultry and animal feeding.

MATERIALS AND METHODS

Materials :

Yeast strain :

The yeast strain used in this investigation was *Candida lipolytica* NRRL-y-1095. This strain was kindly provided from Northern Regional Research Laboratory (NRRL), USA.

Cultivation medium :

The cultivation medium of Anne and Molin (1993) was used for cultivation of *Candida lipolytica* NRRL-y-1095. The yeast extract malt broth medium (NRRL) was used for inoculum preparations and maintenance of the yeast strain.

Wastes used :

Sugar cane molasses (SCM) that obtained from sugar cane factory 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. Corn gluten meal obtained from starch and glucose factory was used as a sole nitrogen source. This waste containing 2.22 % nitrogen. Each of these wastes was replaced with the other source in the cultivation medium used in the same ratio.

Methods :

Preparation of industrial wastes used :

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 Agarual, (1993) with little modification. H_2SO_4 solution was used to reduce the pH value to reach 3.0. Sample was boiled at 100 ^oC 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 corn gluten meal, was oven dried at 105 ^oC for 2 hr. and milled. About 7.5 g of waste was added to 100 ml of 1.5 % H_2SO_4 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 ^oC for different times.

Candida 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^{-1} 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 ^oC; 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 ^oC 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 ^{o}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 :

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

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 ^{o}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, $R^2 = 0.9586$.

Single cell oil extraction :

Oil extraction was carried out according to the method of Granger *et al*. (1993) with little modification as follows: yeast cells were separated by centrifu-gation at 6000 rpm (Type 16000, sponnung 220 V, German Democratic Republic) for 15 min. and dried at 60 ^{o}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 ^{o}C then filtered using a filter paper and oven dried at 70 ^{o}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 :

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

Fatty acids measurement :

The method of extraction for measurement of fatty acids of obtained single cell 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_2SO_4 + 100 ml methanol) + 1 ml benzene were put in a well closed tube. The tube was inserted in water bath at 90 ^oC or in oven at 90 ^oC for 90 min. The tube was allowed to cool then 4 ml distilled H_2O was added + 2.5 ml petroleum ether and shaked well. The ether layer (upper layer) was removed in a small vial and evaporated. Then at injection 50 ml n. hexane was add. The tested sample was injected in HP (Hewlett Packard) 6890 GC instrument with : Detector : FID (Flameionization detector) and Detector temperature : 250 ^oC.

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 *Candida lipolytica* NRRL-y-1095 namely glucose syrup, sugar cane molasses, beet molasses, potato peel, squash and tomatoes peels. Each of these sources was replaced with glucose in the cultivation medium as control in a concentration of 100 gL^{-1} 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 3.9 which recorded with glucose syrup as carbon source. The highest pH values observed with beet molasses being 6.19. For sugar consumed (gL^{-1}), the highest consumed sugar was found when sugar cane molasses was used as carbon sources to be 56.2 gL^{-1} while the lowest sugar consumed was observed in case of squash peel using as carbon source (25.00) comparing to the control value of 34.2 gL^{-1} 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 34.6 gL^{-1} and 5.8 % of γ-linolenic acid from different strains belonging to Mucorales.

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

Waste used (C-source)	Finial pH	Sugar consumed g/L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient	Oil yield efficient (%)	Oil percentage (%)	dx/dt	dp/dt
Glucose syrup	3.90	44.5	3.713	0.95	8.34	2.134	25.55	0.0773	0.0197
Sugar cane molasses	5.42	56.2	5.10	1.35	5.380	2.40	26.470	0.106	0.028
Beet molasses	6.19	39.85	2.061	0.51	5.171	1.279	24.745	0.0429	0.01062
Potato peel	5.30	32.81	2.85	0.45	8.68	3.42	15.789	0.059	0.0093
Squash peel	5.95	25.00	3.00	0.55	12.00	0.022	18.33	0.0625	0.0114
Tomatoes peel	6.09	28.70	3.075	0.308	10.714	1.073	10.0162	0.0640	0.0064
Glucose (control)	5.11	34.2	4.00	0.88	11.69	2.57	22.00	0.0833	0.00183

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

For the cell weight (gL^{-1}) obtained of the used yeast *Candida lipolytica* NRRL-y-1095, sugar cane molasses (SCM) recorded the highest cell weight being 5.10 gL^{-1} (Table 1). On the other hand, beet molasses showed the lowest value of obtained biomass to be 2.061 gL^{-1} compared to that value obtained with control being 4.0 gL^{-1}. The cell weight correlated with oil weight produced gL^{-1} since sugar cane molasses gave the highest produced oil to be 1.35 gL^{-1}. This means that the increase fold equal to 1.28 and 1.5 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 *Candida lipolytica* NRRL-y-1095. Results of Syed *et al*. (2006) proved that glucose was the best carbon source with dry biomass production of 34.6 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 72.41 gL^{-1} recorded in case of 120 gL^{-1}. This result was not related to the cell weight since the growth of *Candida lipolytica* NRRL-y-1095 was 4.783 gL^{-1}. 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 means that the concentration of 120 gL^{-1} of sugar cane as a carbon source was the optimum concentration required for microbial oil production being 1.66 gL^{-1}. This might be due to intolerance of the cells to high concentration of glucose which increase the osmotic potential of the medium.

Table 2: Effect of sugar cane molasses concentration on single cell oil and biomass production.

C-source concent., gL^{-1}	Finial pH	Sugar consumed g/L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient (%)	Oil yield efficient (%)	Oil percentage (%)	dx/dt	dp/dt
80	5.60	50.3	3.338	1.00	6.636	1.988	29.958	0.0695	0.0208
100	5.4	54.8	5.447	1.818	9.939	3.317	33.3761	0.1134	0.0378
120	5.4	72.41	4.783	1.66	6.605	2.43	34.728	0.0996	0.0345
140	4.90	66.15	4.07	1.37	6.152	2.071	33.66	0.0847	0.0285
160	5.4	55.4	6.809	1.59	12.29	2.87	31.610	0.1418	0.03312
180	5.4	67.21	5.00	1.55	7.43	2.306	31.00	0.1041	0.0322

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 NH_4Cl as control. As shown in Table 3, little change in pH value of the

cultivation medium was found up to the lowest values of 4.21 that recorded with corn gluten meal as N-source. For sugar consumption, the highest sugar consumed was 75.2 gL^{-1} that recorded when corn gluten meal used as N-source. The highest yield of biomass to be 5.5 gL^{-1} was also recorded with corn gluten meal that correlated with microbial oil weight produced being 2 gL^{-1}. These results proved that corn gluten meal considered to be the most favourable N-source required for microbial oil production by *Candida lipolytica* NRRL-y-1095.

Table 3: Effect of agri-industrial by-products as N source on single cell oil and biomass production.

Nitrogen source	Finial pH	Sugar consumed g/L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient %	Oil yield efficient %	Oil percentage %	dx/dt	dp/dt
Corn gluten meal	4.21	75.2	5.50	2.00	7.3138	2.65	36.36	0.1145	0.0416
Corn steep Liquer	5.12	45.9	2.11	0.55	4.5969	1.190	26.066	0.0439	0.0114
Protelan	5.01	57.5	5.35	1.81	9.3043	3.14	33.83	0.1114	0.0377
Rice bran	5.24	51.12	2.42	0.65	4.7339	1.27	26.85	0.0504	0.0135
NH_4Cl (Control)	5.11	34.2	1.88	0.42	5.4970	1.169	22.3166	0.0391	0.00875

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

Nitrogen concentration :

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. *Candida lipolytica* NRRL-y-1095 consumed 90.5 gL^{-1} sugar when using 0.130 gL^{-1} N-concentration in the cultivation medium that produced 6.44 gL^{-1} of biomass. The obtained oil weight was equal to 2.57 gL^{-1}. 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. 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 as shown in Table 5. Little change in the final pH values was found between the different treatments. At pH 5.5 treatment, the cell weight of *Candida lipolytica* NRRL-y-1095 reached to 5.5 gL^{-1} with oil weight of 1.75 gL^{-1}. When using initial pH of 6.0, 5.01 gL^{-1} of *Candida lipolytica* NRRL-y-1095 was obtained with 2.11 gL^{-1} of single cell oil. On the other hand, pH 6.5 recorded the highest sugar consumption to be 112.3 gL^{-1}. It was found that lipid 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 3.0-6.0.

Table 4: Effect of N-source concentration on single cell oil and biomass production.

N- source concent., gL^{-1}	Finial pH	Sugar consumed oil g/ 100L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient %	Oil yield efficient	Oil percentage %	dx/dt	dp/dt
0.032	4.91	40.12	5.10	1.55	12.71	3.86	30.39	0.0804	0.0322
0.065	5.14	85.5	5.60	2.13	6.549	2.49	38.03	0.0933	0.0443
0.130	5.11	90.5	6.44	2.57	7.116	2.83	39.75	0.0620	0.0535
0.195	4.98	82.3	6.30	2.41	7.654	2.928	38.25	0.061	0.0502
0.260	5.22	73.5	6.12	2.24	8.326	3.04	36.60	0.1243	0.0466
0.320	5.34	74.6	5.78	1.88	7.640	2.52	32.9	0.1187	0.0391
0.390	5.2	65.1	4.10	1.32	4.10	2.027	32.19	0.0415	0.0275
0.455	4.97	31.5	5.12	1.32	12.962	4.190	25.78	0.0695	0.0275
0.520	4.95	40.1	4.32	1.10	10.773	2.74	25.46	0.0570	0.0229

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

Table 5: Effect of initial pH of cultivation medium on single cell oil and biomass production.

Initial pH	Finial pH	Sugar consumed g/L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient %	Oil yield efficient %	Oil percentage %	dx/dt	dp/dt
4	5.1	34.2	1.175	0.17	3.435	0.49	14.46	0.0163	0.00236
4.5	4.9	39.5	3.330	0.58	8.430	1.46	17.41	0.0462	0.0080
5	4.8	45.6	5.445	1.40	11.951	3.070	25.71	0.0756	0.01944
5.5	5.1	65.5	5.50	1.75	8.396	2.67	31.818	0.0763	0.0243
6	5.2	105.5	5.01	2.11	4.74	2.00	42.11	0.069	0.0293
6.5	5.7	112.3	4.64	1.80	4.1317	1.602	38.79	0.064	0.025
7	5.2	86.2	4.00	1.30	4.6403	1.508	32.5	0.055	0.0180

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 12.7 gL^{-1} that recorded with 15 % NaCl. For cell weight of *Candida lipolytica* NRRL-y-1095, the obtained weight was gradually decreased with increase of NaCl to the lowest value up to 0.83 gL^{-1} with 15 % NaCl. The same trend of negative correlation was also found with oil production since the lowest value of microbial oil obtained was 0.1 gL^{-1} 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 single cell oil and biomass production.

NaCl concentration (%)	Finial pH	Sugar consumed g/L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient %	Oil yield efficient %	Oil percentage %	dx/dt	dp/dt
1	5.2	38.9	4.320	1.65	10.854	4.89	38.19	0.06	0.0229
3	5.7	38.5	3.85	1.44	10.00	4.025	37.40	0.0534	0.02
5	4.9	33.20	3.614	1.10	10.885	3.614	35.48	0.0501	0.0152
7	4.1	22.50	2.73	0.89	12.133	3.95	32.60	0.0379	0.0123
10	5.00	14.78	0.95	0.16	6.427	1.082	16.84	0.0131	0.0022
15	3.5	12.7	0.83	0.10	6.535	6.25	12.04	0.01152	0.0013
Control	5.22	83.6	6.34	2.48	7.583	2.266	39.116	0.1320	0.0516

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 *Candida lipolytica* NRRL-y-1095. As shown in Table 7, the treatment of 20 ^{o}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 101.2 gL^{-1}. This was correlated with the highest production of either cell weight or oil weight to be 6.9 gL^{-1} or 2.95 gL^{-1} for cell weight or oil weight, respectively. The growth of tested yeast was low at temperature below 20 ^{o}C and above 30 ^{o}C as illustrated in Table 7. Patricia *et al*. (1999) found that 28 ^{o}C was the optimum temperature for biomass production as well as lipid content production. They obtained 2.47, 5.83 and 4.29 gL^{-1} biomass of *Mucor sp* LB-54 at 12, 28 and 38 ^{o}C, respectively. At the same temperature, the production of lipid content was 0.39, 1.21 and 0.49 gL^{-1}, respectively.

Table 7. Effect of growth temperature on single cell oil and biomass production.

Temp. ^{o}C	Finial pH	Sugar consumed g/L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient	Oil yield efficient %	Oil percentage %	dx/dt	Dp/dt
15	5.4	55.5	3.81	1.22	7.00	2.198	32.02	0.079	0.02
20	5.2	101.2	6.90	2.95	7.00	2.915	42.75	0.143	0.06
25	5.6	94.5	4.911	1.98	5.00	2.09	40.30	0.102	0.04
30	5.4	91.2	4.71	1.85	5.00	2.02	39.20	0.098	0.03
35	5.5	73.2	4.291	1.44	6.00	1.967	33.55	0.089	0.03
40	5.3	35.4	2.201	0.35	6.00	0.988	15.901	0.045	0.00

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

Incubation period :

The behaviour of *Candida lipolytica* NRRL-y-1095 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 72 h to be 97.15 gL^{-1}. After 72 h incubation the biomass weight was 6.35 gL^{-1}. For oil weight production, data showed that 2.66 gL^{-1} of single cell oil was produced after 72 h of incubation at 20 ^{o}C.

Table 8: Effect of incubation period on single cell oil and biomass production.

Incubation period, hr	Finial pH	Sugar consumed g/L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient %	Oil yield efficient %	Oil percentage %	dx/dt	dp/dt
24	5.40	55.5	4.00	1.35	7.20	2.432	33.75	0.083	0.0281
48	5.22	83.6	6.34	2.48	7.583	2.966	39.116	0.1320	0.0516
72	5.30	97.15	6.35	2.66	6.5362	2.738	41.88	0.1322	0.0554
96	5.6	62.4	5.95	2.01	9.5352	3.22	33.7815	0.1239	0.0418
120	5.6	58.3	5.75	1.82	9.86	3.121	31.65	0.119	0.0379

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 *Candida lipolytica* NRRL-y-1095 used sugar in increase ratio with the increase of agitation used. The increase of sugar consumption reached to the highest value to be 113.2 gL^{-1} 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 5.2 gL^{-1} that recorded in case of 200 rpm. Again data of bio-oil produced by the tested yeast showed also gradual increase up to 2.25 gL^{-1} 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 (*Candida lipolytica*), 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 single cell oil and biomass production.

Agitation speed, rpm	Finial pH	Sugar consumed g/L	Cell weight gL^{-1}	Oil weight gL^{-1}	Growth yield efficient%	Oil yield efficient %	Oil percentage %	dx/dt	dp/dt
100	5.2	50.3	1.210	0.402	2.405	0.799	33.22	0.016	0.0055
125	5.6	58.5	2.45	0.921	4.188	0.1574	37.59	0.0340	0.0127
150	4.6	74.2	3.224	1.34	4.345	1.805	41.56	0.044	0.0186
175	4.5	104.5	4.61	1.95	4.4114	1.866	42.29	0.064	0.0270
200	4.3	113.2	5.20	2.25	4.593	1.98	43.26	0.0722	0.03125

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

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تحويل جلوتين الذرة إلى زيت ميكروبي بإستخدام خميرا الكانديدا

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جلوتين الذرة هو مخلف لمصنع النشا والجلوكوز وخميرة الكانديدا من الخمائر القادرة على تصنيع الزيت الميكروبي على بيئة تحتوى على مخلف زراعي صناعي مثل مولاس قصب السكر كمصدر وحيد للكربون وجلوتين الذرة كمصدر وحيد للنتروجين ولقد تمت دراسة الظروف المثلي لإنتاج الزيت الميكروبي والكتلة الحيوية وبينت النتائج المتحصل عليها أن أنسب الظروف لإنتاج الزيت الميكروبي هي 72 ساعة ، 20 °م ، 5.5 ، 200 لفة وذلك لكل من فترة التحضين ، درجة الحرارة ، الأس السالب لتركيز أيون الأيدروجين الخاص ببيئة الزرع 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 *Candida lipolytica* NRRL-y-1095 لإنتاج الزيت الميكروبي بإستخدام بعض المخلفات الزراعية المتخلفة عن العمليات الصناعية وذلك من وجهة النظر الإقتصادية وكذلك لحماية البيئة.