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Glucose utilization and lipid production by oleaginous yeast cultures

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The laboratory experiment was conducted to investigate the effect of different concentrations of glucose ranges from 0.11 to 0.66 M and utilization pattern (7 days) was studied for lipid production in oleaginous yeast cultures. Lipid and biomass production was gradually increased in response to glucose concentration. In *Rhodotorula glutinis, Rhodosporidium toruloides* and *Lipomyces starkeyi* lipid content reached maximum as 4.55 g l^{-1} (40.26 per cent), 4.25 g l^{-1} (38.20 per cent) and 4.23 g l^{-1} (38.10 per cent), respectively at 0.55 M of glucose concentration. Biomass content was also high as 11.30, 11.13 and 11.10 g l^{-1}, respectively at 0.55 M concentration. While studying the utilization pattern, consumption of glucose was started from the second day with small amount of lipid and biomass production and it was gradually increased on third and fourth days of fermentation period. Maximum amount of lipid (4.80, 4.64 and 4.61 g l^{-1}) and biomass (11.40, 11.26 g, 11.20 g l^{-1}) was recorded on fifth day of fermentation with the utilization of 0.5 M of glucose. Then decline in lipid production was observed on sixth and seventh days. At the end of fermentation *Rhodotorula glutinis* utilized 0.55 M of carbon and exhibited 4.56 g l^{-1} of lipid and 11.30 g l^{-1} of biomass.

Key words : Oleaginous yeast, Lipids, Biomass, Carbon source, Glucose utilization

INTRODUCTION

Lipid accumulation is a dynamic process, which depends on the microorganism, the growth conditions and the growth phase. Most oleaginous microorganisms start to accumulate oil whenever excess carbon source is present. While, at the same time, growth is limited by another nutrient (Ratledge and Evans, 1989). Depending on the microbial species and environmental conditions, the lipid content of microorganisms may vary between a few per cent to over 80 per cent of the biomass dry weight (Ratledge, 1993; Leman, 1997).

Lipid accumulation in an oleaginous microorganism begins when it exhausts a nutrient from the medium, usually this is nitrogen but with a surfeit of carbon, usually in the form of glucose, still remaining. Glucose continues to be assimilated by the cells and is converted into triacylglycerols at more or less the same rate at which lipid was synthesized during the balance phase of growth. However, the limitation in the supply of nitrogen arises, the cell proliferation is prevented, and the lipid that is now formed has to be stored within the existing cells which can no longer divide (Ratledge, 2002). Lipids serve as storage materials in some lipid accumulating yeasts, e.g. *Rhodotorula graminis*. It is reported that yeasts can store up to 70 per cent of lipids in dry matter (Guerzoni *et al*., 1985).

Most yeasts produce small numbers of cytosolic lipid bodies, but the oleaginous yeasts can accumulate up to 25 per cent (w/w) storage lipid in response to a high carbon: nitrogen (C/N) ratio (Leber *et al*.,1994). Lipid bodies in *Saccharomyces cerevisiae* contain almost equal amounts of TAGs and sterol esters. Studies of Leber *et al*. (1995) imply that, in yeast, lipid bodies do not serve simply as inert lipid stores but play an important role in the biosynthesis, mobilization and trafficking of intracellular neutral lipids. Hence, this present study was undertaken to optimize the glucose concentration for increasing the lipid production in oleaginous yeast cultures.

MATERIALS AND METHODS

Laboratory experiments were conducted in the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore (T.N.) in the year 2008 to study the influence of varied concentrations of glucose on biomass as well as lipid production by oleaginous yeast cultures. The pattern of glucose utilization for lipid production was also assed.

Influence of levels of glucose on lipid and biomass production :

Oleaginous yeast cultures *viz., Rhodotorula glutinis* (MTCC 247), *Rhodosporidium toruloides* (MTCC 1400) and *Lipomyces starkeyi* (MTCC 2974) were collected from MTCC, Chandigarh, India and used for the experiment.

Screening broth (Dai *et al.,* 2007) containing yeast extract -15.0g,/l, Peptone 5.0g/l was prepared without carbon source, in which different levels (0.11 M to 0.66 M) of glucose was added separately. The pH of the broth was adjusted to 6.0 and three replications were maintained

for each carbon source. The broth was inoculated with 24 h old cultures grown in yeast extract peptone glucose broth at 10 per cent level containing $28x10^4$ cfu/ml in the broth. (Saxena *et al*., 1998).

Inoculated flasks were incubated at 30^0C for 7 days in an incubator shaker at 200 rpm (Innova 4320, New Brunswick, USA) for the growth of culture. After seven days of growth, cultures were harvested by centrifugation and the cell pellets were obtained. Lipid content and biomass production of dried yeast cells were estimated by adopting the standard methods.

Cell biomass determination :

For dry weight determination, culture sample was centrifuged at 6000 rpm for 10 min at room temperature and cell pellet was washed first with 0.1 M phosphate buffer at pH 7.0. The supernatant was discarded and the cell pellet was dried at 65^0C for 48 h. After cooling, the cell dry weight was estimated (Esther *et al*., 1988).

Extraction of yeast lipid :

Yeast lipid was extracted from the dried cells as described by Bligh and Dyer (1959) using methanol chloroform mixture 2:1 (v/v). The filtrate was transferred to a graduated glass cylinder. Small quantities of yeast cells remaining in the blender and in the filter paper were washed with 10 ml of chloroform and the contents were transferred to the graduated cylinder and allowed for few min for the phases to separate. The lower volume of chloroform layer (20 ml added above) was recorded as 'x' ml. Upper methanol - water layer was removed by pipetting out along with layer of chloroform to ensure complete removal of methanol-water layer. Again the remaining lower volume of chloroform layer was recorded as 'y' ml. This volume was transferred quantitatively into a pre-weighed conical flask denoted as 'a' g. The contents in the conical flasks were evaporated at 40-50^0C in water bath under stream of nitrogen gas. Remaining residues were cooled and dried over phosphoric anhydride in a vaccum desiccator. Weight of the conical flask was taken again and denoted as 'b' g.

Five ml of chloroform was added three times to dissolve the evaporated lipid. Again flasks were evaporated and dried as above. Weight of the flask was taken at third time and denoted as 'c' g. Amount of lipid present in the yeast cells was calculated by using the following formula:

Weight of lipid (g) = (b-a) - (c-a) ='d' g.

where a - is the weight of empty flask.

$$\text{Total lipid (g)} = \text{Weight of lipid (d)} \times \frac{\text{Total volume of chloroform layer (x ml)}}{\text{Volume of chloroform layer evaporated (y ml)}}$$

$$\text{Total lipid (per cent)} = \frac{\text{Total lipid (g)}}{\text{Weight of sample (g)}} \times 100$$

Lipid production and glucose utilization by oleaginous yeast cultures :

In the earlier study, lipid production was found higher with the glucose concentration of 100g/l (*i.e*. 0.55M) in the broth. This experiment was conducted to find out the relationship between the rate of depletion of glucose source and the formation of lipid in yeast cultures.

The screening broth with 0.55 M of glucose concentration was prepared in 250 ml Erlenmeyer flasks and sterilized. Ten per cent inoculum containing $28x10^4$ cfu/ml of different yeast cultures was inoculated and incubated in environmental shaker at 200 rpm for 7 days. After incubation, culture sample was centrifuged and the cell pellet was used for estimation of biomass and lipids. The filtrates were analyzed for glucose content (Miller, 1972) using arsenomolybdate reagent.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below:

Effect of different concentrations of glucose on lipid production by three oleaginous yeast cultures :

Concentration of glucose required for maximum lipid and biomass production in yeast cultures was studied. The broth was prepared with different glucose concentration ranged from 0.11 to 0.66 M. Lipid and biomass production was gradually increased in response to glucose concentration. In *Rhodotorula glutinis*,*Rhodosporidium toruloides* and *Lipomyces starkeyi* lipid content reached maximum as 4.55 g l^{-1} (40.26 per cent), 4.25 g l^{-1} (38.20 per cent) and 4.23 g l^{-1} (38.10 per cent), respectively at 0.55 M of glucose concentration. Biomass content was also high as 11.30, 11.13 and 11.10 g l^{-1}, respectively of *Rhodotorula glutinis, Rhodosporidium toruloides* and *Lipomyces starkeyi* at 0.55 M concentration. There after lipid production was declined to 38.30 per cent in *Rhodotorula glutinis*, 36.40 per cent in *Rhodosporidium toruloides* and 35.50 per cent in *Lipomyces starkeyi*, respectively at 0.66 M concentration.

When the mean values of the cultures were

compared, biomass and lipid content was higher (11.17 g l^{-1} and 4.34 g l^{-1}) at 0.55M glucose concentration, against the lower biomass and lipid content (9.15 g l^{-1} and gl^{-1}) at 0.11M concentration. Among the three cultures, *Rhodotorula glutinis* produced higher biomass (10.57 g l^{-1}) and lipid (3.24 g l^{-1}). Similarly 0.55M and 0.66M carbon yielded best results for lipid and biomass and they were at par with each other. With the increase in glucose concentration, biomass production and lipid accumulation was significantly increased up to 0.55 M concentration after that started declining, which is an agreement with the results observed by Saxena *et al*. (1998).Similar results also reported in other oleaginous yeast strains as 22-25 gl^{-1} (Bail *et al*., 1984), 5.2 gl^{-1} (Colin *et al*., 1977) of lipids produced with of 100g glucose consumption (*i.e*. 0.55M). While further increase in the glucose concentration (0.66 M) resulted decreased biomass and lipid accumulation. In general no significant differences among the cultures were noticed with respect to either biomass, or lipid yield. But concentration of glucose exhibited significant variation. Similarly lipid in the biomass did not show significant variation among the cultures as well as with higher concentration of glucose (Table 1 and Fig. 1).

At lower concentration of glucose lipid content of the yeast was less, probably due to diversion of the glucose to energy production. Under nitrogen limiting conditions with the presence of a carbon-source in excess, organisms started to store lipids. Therefore, a high carbon to nitrogen (C/N)-ratio, around 100, is a basic requirement for the accumulation of lipids (Holds worth and Ratledge, 1988). The reason behind that yeast strains can tolerate to high glucose concentration.The yeast *Rhodosporidium toruloides* grew well on glucose as a sole source of carbon and energy at a concentration reached up to 150 $gl^{-1.}$ When the glucose concentration reached200gl^{-1} cell growth was greatly repressed due to lack of nitrogen for the synthesis of proteins and nucleic acids and more severe inhibiting effects were observed at higher concentration of glucose (Li *et al*., 2007).

Lipid production and glucose utilization by three oleaginous yeast cultures:

Carbon utilization pattern and lipid production by yeast cultures were determined through out the fermentation period (7 days). In *Rhodotorula glutinis,* glucose consumption started from the second day (0.17)

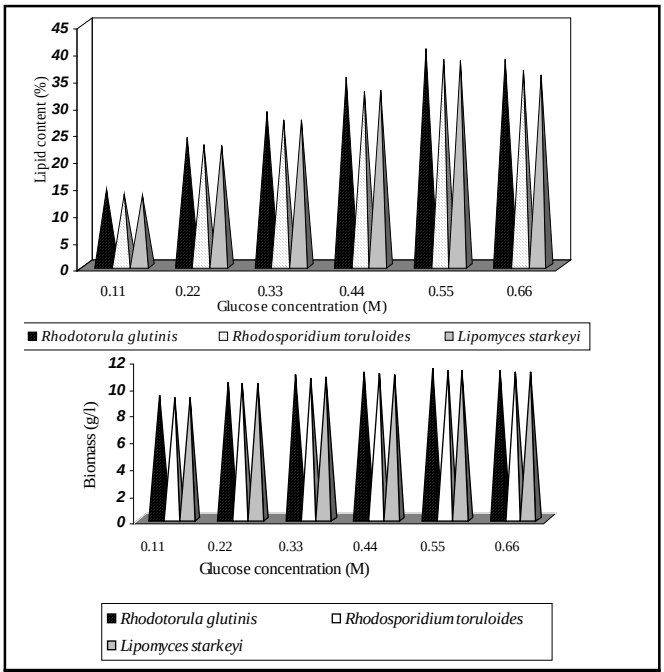

Fig. 1 : Effect of glucose concentrations on lipid production by three oleaginous yeast cultures

Table 1 : Effect of glucose concentrations on lipid production by three oleaginous yeast cultures

Glucose	Biomass (g l^{-1})				Lipid yield (g l^{-1})				Lipids in biomass (%)			
concentration (M)	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
0.11	9.26	9.10	9.10	9.15	1.31	1.20	1.19	1.23	14.13	13.20	13.10	13.47
0.22	10.20	10.15	10.13	10.16	2.42	2.28	2.25	2.31	23.76	22.46	22.20	22.80
0.33	10.80	10.55	10.63	10.66	3.10	2.86	2.88	2.91	28.66	27.20	27.10	27.65
0.44	11.00	10.90	10.80	10.90	3.84	3.54	3.52	3.63	34.90	32.45	32.60	33.31
0.55	11.30	11.13	11.10	11.17	4.55	4.25	4.23	4.34	40.26	38.20	38.10	38.85
0.66	11.10	11.05	11.00	11.05	4.25	4.02	3.91	4.06	38.30	36.40	35.50	36.73
Mean	10.57	10.48	10.40		3.24	3.02	2.99		30.00	28.31	28.10	
	S.E. ±		C.D. (P=0.05)		S.E. ±		C.D. (P=0.05)		S.E. ±		C.D. (P=0.05)	
C	0.19		NS		0.21		NS		1.71		NS	
T	0.27		0.56		0.29		0.59		2.41		4.83	
C X T	0.47		NS		0.51		NS		4.18		NS	

C_1-*Rhodotorula glutinis* C_2- *Rhodosporidium toruloides* C_3- *Lipomyces starkeyi* C- Cultures T- Glucose concentration
NS = Non significant

with small amount of lipid 0.70g l^{-1} and biomass (4.00g l) production and it was gradually increased on third and fourth days of fermentation period. Maximum amount of lipid (4.80 g l^{-1}) and biomass (11.40 g l^{-1}) was recorded on fifth day of fermentation with the utilization of 0.5 M of glucose. Then decline in lipid production was observed on sixth and seventh days. At the end of fermentation *Rhodotorula glutinis* utilized 0.55 M of carbon and exhibited 4.56 g l^{-1} of lipid and 11.30 g l^{-1} of biomass. In *Rhodosporidium toruloides* glucose consumption commenced from the second day (0.15 M) with small amount of lipid and biomass production. The lipid production was gradually increased on third and fourth days of fermentation period. Maximum amount of lipid and biomass was recorded on fifth day of fermentation by utilizing 0.48 M of glucose. It utilizes 0.55 M of carbon at the end of fermentation and exhibited comparatively less amount of lipid (4.27 g l^{-1}) and biomass (11.15 g l^{-1}) than *Rhodotorula glutinis*. Similar trend was observed in which *Lipomyces starkeyi* also recorded maximum lipid yield (4.61 g l^{-1}) on fifth day. At the end of fermentation period, (7 days) lipid and biomass content were recorded lower. (Table 2 and Fig. 2).

A high lipid content of 54 per cent (w/w) was obtained in *Lipomyces starkeyi* at 140 h of fermentation period (Yamachui *et al*., 1983). Generally growth and lipid production were good in aerated glucose solutions containing small amounts of yeast extract as the nitrogen source. Under favourable conditions, 20 to 25 per cent of the consumed glucose was converted to yeast cells which contained 50 to 63 per cent of lipid. About 10 to 14 per cent of the consumed glucose was recovered as lipid. In a fed batch mode, with a C: N ratio of 30, *Rhodotorula*

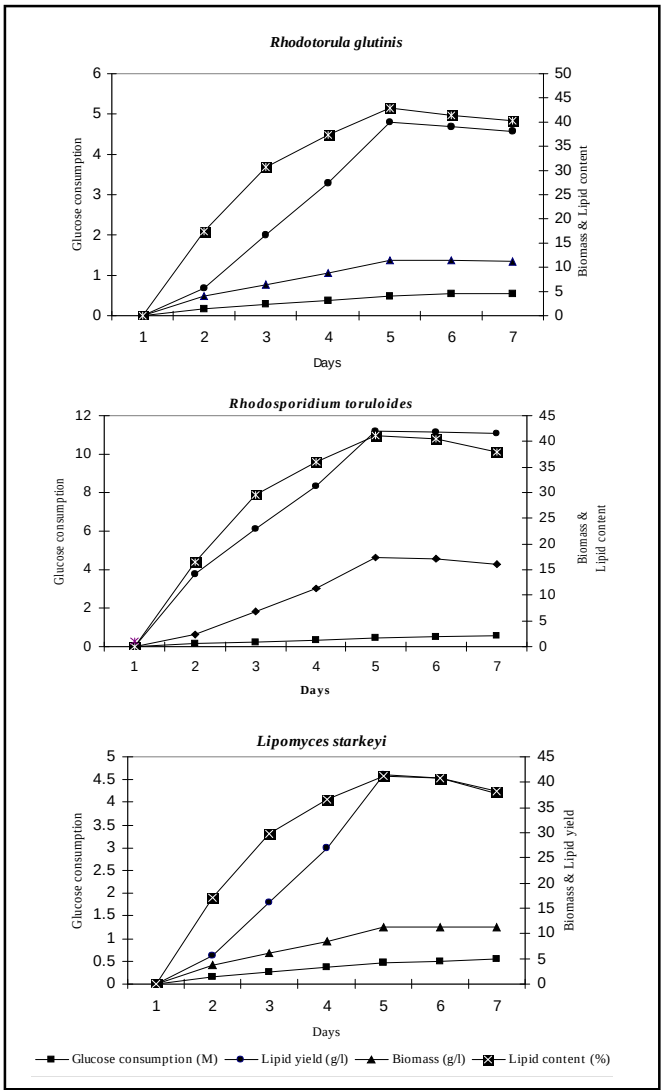

Fig. 2 : Lipid production and glucose utilization by three oleaginous yeast cultures

Table 2 : Lipid production and glucose utilization by three oleaginous yeast cultures

Time	Glucose consumed (M)				Biomass (g l^{-1})				Lipid yield (g l^{-1})				Lipids in biomass (%)			
(Days)	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.17	0.15	0.15	0.16	4.00	3.82	3.80	3.87	0.70	0.65	0.63	0.66	17.50	17.00	16.57	17.01
3	0.28	0.25	0.24	0.26	6.50	6.12	6.10	6.24	2.00	1.82	1.80	1.87	30.76	29.70	29.50	30.00
4	0.38	0.36	0.35	0.36	8.85	8.35	8.32	8.50	3.30	3.05	3.00	3.11	37.28	36.52	36.00	36.60
5	0.50	0.48	0.48	0.48	11.40	11.26	11.20	11.28	4.80	4.64	4.61	4.67	42.90	41.20	41.15	41.75
6	0.53	0.50	0.50	0.51	11.35	11.20	11.15	11.20	4.70	4.56	4.52	4.55	41.40	40.71	40.53	40.85
7	0.55	0.55	0.55	0.55	11.30	11.15	11.10	11.16	4.56	4.27	4.20	4.32	40.17	38.30	37.83	38.76
Mean	0.34	0.76	0.32		7.62	7.41	7.38		2.86	2.71	2.68		30.00	28.90	28.78	
	S.E ±		C.D. (P=0.05)		S.E ±		C.D. (P=0.05)		S.E ±		C.D. (P=0.05)		S.E ±		C.D. (P=0.05)	
C	0.03		NS		0.15		NS		0.19		0.57		2.55		NS	
T	0.05		0.11		1.09		2.21		0.43		0.87		3.59		7.86	
C X T	0.09		NS		1.89		NS		0.75		1.51		6.74		NS	

NS = Non significant

C_1-*Rhodotorula glutinis* C_2- *Rhodosporidium toruloides* C_3- *Lipomyces starkeyi* C- Cultures T- Time (Days)

minuta IIP- 33 recorded the lipid yield of 0.48 and the decline in lipid yield was observed with to increase in C:N ratio to 40 (Saxena *et al*., 1998). Similar results were obtained in the present study, in which increase in glucose level with the similar nitrogen levels (high C: N) results in the reduction. Thus, the excess carbon is not favourable for lipid accumulation, which is also in agreement with the earlier results observed by Patel *et al*. (1992) and Chen and Chang (1996).

These results highlighted the significance of glucose on lipid production, in which 0.55 M glucose resulted better lipid production. While observing the glucose consumption pattern, lipid accumulation was found higher in 5th day of fermentation with utilization of 0.5M of glucose, this may be explained due to the presence of more number of viable cells on 5th day combines with the production of secondary metabolites. There after reduction in biomass results in reduction of lipids on 7th day.

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