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Study of *Rhodotorula glutinis* growth and lipid production using low cost substrates

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ABSTRACT

The present day challenge for biodiesel production is that as feedstock varied vegetable oils such as soybean oils, rap seed oils, palm oils and waste cooking oils are being used making it uneconomical. Microbial oil could represent an alternative to produce bio-oils for biodiesel production. In the present study in our lab red oleaginous yeast *Rhodotorula glutinis* was used for lipid production using various low cost substrates as sole carbon source. *Rhodotorula glutinis* culture was obtained from NCIM Pune and was subcultured and maintained on YPD medium. The growth conditions were optimised and dry cell weight and lipid content were estimated using fruit peel and sugar cane residue hydrolysate. The study can be further extended using different other substrates and result in cost effective high density cell culture.

Key words: biodiesel, microbial lipid, low cost substrate, *Rhodotorula glutinis*.

INTRODUCTION

Some yeast strains, such as *Rhodosporidium* sp., *Rhodotorula* sp. and *Lipomyces* sp. can accumulate intracellular lipids as high as 70% of their biomass dry weight. The majority of those lipids are triacylglycerol containing long-chain fatty acids that are comparable to conventional vegetable oils.(Ratledge & Wynn, 2002). More importantly, some of those oleaginous species show the ability to metabolize pentoses, demonstrating the potential to produce TAG from lignocellulosic biomass and other cheap materials (Meesters et al., 1996, Papanikolaou et al., 2002). Recent demand for biodiesel worldwide has turned TAG into an ever-growing and substantial consumption resource(Easterling et al., 2009, Angerbauer et al., 2012), The basic physiology of lipid accumulation in microorganisms has

been well studied. It is known that lipid production requires medium with an excess of sugars or similar components (e.g., glycerol, polysaccharides, etc.) and limited other nutrients, usually nitrogen. Thus, oleaginous potential is critically affected by the carbon-to-nitrogen (C/N) ratio of the culture and other factors like aeration, inorganic salt presence, etc.The costs of microbial oil production are currently higher than those of vegetable oil but there are many methods to drastically improve the techno-economics of microbial oil production processes. In particular, the exploration of lignocellulose-based carbohydrates as feedstock may greatly lower the costs (Pirrozi et al., 2012)In this study, we have studies the growth of *Rhodotorula glutinis* and the lipid production with low cost substrates such as whey and fruit peel juice. The studies are important due to the economic importance of this yeast as potential strain for lipid production to be used in biodeisel production.

2. MATERIALS AND METHODS

2.1. Organism, media and chemicals

Rhodotorula glutinis culture was obtained from NCIM Pune and was subcultured for maintenance on YPD slants using slant streak method.

Yeast extract (0.5%) was prepared in basal media of composition 0.3% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 5% inoculum was added and incubated at different temperatures and pH.

The experiments were carried out to check the growth at varying pH and temperature.All other chemicals and reagents were bought locally and were of analytical reagent grade.

2.2. Biomass yield

Five gram yeast were inoculated in 100 ml of YPD medium and shook with 120rpm for more than 12 hrs. Ten millilitres of cultured yeast was transferred in to a test tube and centrifuged with 4500 rpm for 20 minutes. Supernatant was removed and the pellet was collected and inoculated into 5 % (w/v), 10 % (w/v) and 15 % (w/v) in the above mentioned basal media.

Yeast growth was measured every two hrs for 14 hrs at 540 nm wave length. Based on the data the microbial growth curves were plotted for each strain.

2.3. Analytical method used for measuring and analyzing yeast growth

Yeast growth kinetics

Five ml of cultured medium sample was taken in every 12 hours time interval until the 36solution optical density was measured at 540 nm wave length. A blank solution was used for reference reading. The results were recorded and used in plotting growth curve of the yeast cells.

Biomass estimation

At the end of the yeast growth, the biomass was harvested centrifuging the culture medium at 4500 rpm for 20 minutes. During centrifuging the supernatant and pellet was separated. The remaining pellet was diluted with 5 ml distilled water and its optical density was measured at 540 nm.

Dry weight determination

The wet cells were collected by centrifugation and washed with the same volume of distilled water. Cell dry weight was obtained from wet

cells from a 20 ml culture broth after being dried at 105 æ% C overnight.

Lipid extraction

The lipid was extracted by the method of Soxlet method, 1995. The lipid content was expressed by the percentage of lipid in the DCW.

Treatment of fruit peel

The fruit peels were dried, ground and hydrolysed as per Wang el al.

3. RESULTS

3.1. Effect of different Substrate concentration

To determine the effect of substrate concentration on growth profile of *R. glutinis* batch experiments were performed in conical flasks with glucose concentration ranging from 10 to 400 g/ l. Table 1 shows the plots of cell culture optical density against fermentation time. These results indicated that the yeast grew well on glucose as a sole source of carbon and energy at a concentration up to 150 g /l. When the glucose concentration reached 200 g/ l, cell growth was greatly repressed and more severe inhibitory effects were observed at even higher glucose concentrations.

These data suggested that a wide range of substrate concentrations were suitable for growth of this strain with no significant substrate inhibition. However, drastic growth rate loss was found for the culture when glucose concentrations were higher than 200 g/ l.

3. 2. Effect of different pH

To determine the effect of pH on growth profile of *R. glutinis* batch experiments were performed in conical flasks with pH ranging from 5 to 8. Fig 2 shows the plots of cell culture optical density against fermentation time. These results indicated that the optimum pH for this strain was 6 with no growth at pH 8. Correspondingly the lipid content was also found to increase.

Table 1.

Time	Optical Density				
	10g/l	50g/l	100g/l	200g/l	400g/l
0	0	0	0	0	0
2	0.1	0.1	0.1	0.05	0.05
4	0.15	0.2	0.15	0.1	0.06
6	0.2	0.3	0.2	0.12	0.06
8	0.25	0.4	0.25	0.15	0.06
10	0.3	0.5	0.3	0.16	0.06
12	0.35	0.6	0.35	0.18	0.06

Effect of initial glucose concentration on growth of *Rhodotorula glutinis.*

Table 2

Carbon Source	Glucose	Pomegranate peel	Sugar cane dust
Dry cell weight	13.4 g/l	7.2 g/l	10.2 g/l
Lipid content	1.2 g/l	0.6 g/l	9.8 g/l

Dry cell weight and Lipid Content (12 hrs) using different substrate as sole carbon source.

3.3. Effect of different Substrates

The growth pattern was also observed using fruit peel of pomegranate and sugar cane residue after juice extraction as substrate. It was observed that the yeast was able to show growth in the absence of glucose when these substrates were used. The growth was maximum for sugar cane residue and was less for pomegranate under all other similar conditions.

4. DISCUSSION

Since *Rhodotorula glutinis* is one of the yeast strains that can accumulate intracellular lipids as high as 70% of their biomass dry weight, in an effort to achieve a high density cell culture, this study was carried out. The fruit peel of pomegranate and sugar cane which is a waste product of fruit juice industry were found to have potential to act as low cost substrates for biomass production of this yeast. Wang *et al*., has found similar results with the oleaginous yeast *Lipomyces starkeyi* utilizing diverse carbon sources including glucose, xylose, glycerol, and willow wood sawdust (WWS) hydrolysate for the biosynthesis of oils and the cell growth. The present study can economise the production of microbial lipid which is being considered as an important raw material for bio-diesel production. The soxlet method has its disadvantage that only extractable lipids could be estimated but nevertheless it gave a general idea of the relationship between the biomass and lipid content(Li et al.(2007), Zhao et al.(2005)).This study can be extended to further study the use of other lingo-cellulosic waste in achieving a high density cell culture of this economically important yeast.

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