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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Comparative Study on *Candida Sp*. for the Production of Glycerol

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Abstract : *Candidmagnoliae* and *Candida glycerinogenes* are the yeast isolated from the natural environment used for the production of glycerol. In this work potential of glycerol production by both *candida* species was compared by the fermentation of glucose by optimizing certain parameters.*C.glycerimogenes*converts up to 64.5% (w/w) of the available glucose into glycerol and it was 55% in C. *magnolia*. *C. glycerinogenes*yields 9% of higher concentration of glycerol than *C. magnoliae.* In *C. magnoliae*the optimum temperature of 30°C and a pH of 5 yields highest glycerol production whereas for *C.glycerinogenes*a temperature of 32°C and a pH of 5. The parameters which found to influence the glycerol production like phosphate ,glucose were studied.
Key words :*Candidmagnoliae,Candida glycerinogenes,*glycerol.

Introduction

Glycerol, a simple alcohol which contains three hydroxyl group. Glycerol is also known as glycerine or 1,2,3-Propanetriol. Glycerol has many uses in pharmaceutical, food, paint, cosmetic industries[1]. Glycerol can be used additional fuel in boilers due to its high calorific value. The physiochemical properties and chemical composition of glycerol varies from other fuels[2].

Glycerol is aodourless, colourless, viscous liquid with sweet taste. Glycerol contains three hydrophilic hydroxyl groups which is responsible for its solubility in water. Glycerol has a melting point of 17.9°C and boiling point of 290°C. Molecular formula of glycerol is $CH_2OH\text{-}CHOH\text{-}CH_2OH$.[3]. Glycerol can be transformed to various value added chemicals such as dihydroxyacetone, succinic acid, citric acid, ethanol, hydrogen etc., Until now the fermentative metabolism of glycerol was being reported in species of bacteria like *Citrobactersp, Enterobactersp, Lactobacillus sp, Propionibacteriumsp, Clostridium* and many fungi species.

Glycerol has become an abundant carbon source and inexpensive. Glycerol is also produced by yeast fermentation process. Glycerol is obtained as a byproduct during the fermentation of sugar to ethanol using *Saccharomyces cerevisiae*[4]. Increased glycerol production from monosaccharide's can be obtained using yeast fermentation[5].

The production of glycerol in the laboratory is possible by yeast *Candida magnoliae* and osmotolerant yeast *Candida glycerinogenes.*Using genetic information, there are new possibilities in the field of fermentation and metabolic engineering[6]. The Overexpression or blocking of genes could potentially can increase yield or productivity. Triose phosphate isomerase is an important enzyme in glycolytic pathway that directs dihydroxyacetone phosphate to glyceraldehyde 3-phosphate. When this triose phosphate isomerase gene was deleted, the mutant is able to achieve higher yield of glycerol[1]. Overexpression of GPD1 gene in yeast increases glycerol production simultaneously increases the accumulation of byproducts such as succinate, acetate, pyruvate etc.

This work briefs about the comparison of glycerol production in *Candidamagnoliae*and *Candidaglycerinogenes.*For the design of fermentation process, culture media optimization is an essential step. Many parameters such as phosphate, sulfate, temperature and pH have been found to affect the productivity of glycerol by these microorganisms. Hence these were optimized in prior to other parameters.

Materials and Methods

Organisms and Media

All fermentation procedures were carried out with both *C. magnoliae*and *C. glycerinogenes*.*C. magnoliae*cells were propagated in medium containing 3 g/L of yeast extract, 160 g/L of glucose, 3 g/L of malt extract, 5 g/L of peptone. *C. glycerinogenes* cells were propagated in medium containing 150 g/L of glucose, 2 g/L of urea and 7 ml/L of corn steep liquor. YEP medium is required to grow *C. glycerinogenes*and YM medium is required to grow *C. magnoliae*. The cells were grown until the density reaches 0.2 OD[7,8].

Fermentation Process

Fermentations were carried out in 250 ml shake flasks with a working volume of 50 ml. To the working medium 5% (v/v) of *C. glycerinogenes and C. magnoliae*were inoculated in different flasks. Flask for *C. glycerinogenes* was incubated at 31 ^{o}C and flask for *C. magnoliae*was incubated at 30 ^{o}C for 48 hours. The medium was agitated at 500 rpm[9,10].

Results

Candida magnolia and *C. glycerinogenes*were isolated from the natural environment. Both the microorganisms were capable of utilizing glucose as a carbon source for the growth and strongly ferment glucose to glycerol. This yeast is able to grow in YEP and YM medium containing glucose, yeast extract, malt extract and peptone.

Optimization

Effect of Glucose Concentration on Glycerol Production

Table.1: Effect of different concentration of glucose on glycerol productivity

Glucose (g/L)	Glycerolfor *C. magnolia*(g/L)	Glycerol for *C. glycerinogenes* (g/L)
0	40.9	99.8
100	45.3	104.1
130	49.1	110.3
160	52.6	116.7
190	45.0	120.9
210	41.2	123.2
240	38.5	117.4
270	34.6	106.8

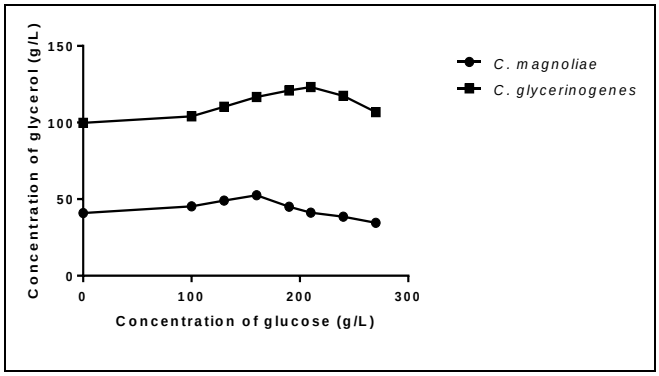

Fig. 1: Effect of initial concentration of glucose in the medium on the production of glycerol by *C.magnoliae*and *C. glycerinogenes*based on the amount of glucose consumed.

The effect of different concentration of glucose for the glycerol production by *C.magnoliae*and *C. glycerinogenes* was determined in 250 ml flask containing 50 ml of medium. For *C. magnoliae*, concentration of glycerol yield increases when the concentration of glucose was increased from 100 g/l to 200 g/l and for *C. glycerinogenes*, concentration of glycerol increases when the concentration of glucose ranges between 150 g/L to 250 g/L. Further increase in the concentration of glucose causes a remarkable decrease in the yield. The optimum concentration of glucose for the growth using *C. magnoliae*was found to be 160 g/L and for *C. glycerinogenes*, glucose concentration was found to be 210 g/L.

Effect of Phosphate Concentration on Glycerol Production

Table 2 Effect of different concentration of phosphate on glycerol productivity

Phosphate (g/L)	Glycerol for *C. magnoliae* (g/L)	Glycerol for *C.glycerinogenes* (g/L)
0	45.8	76.2
2	46.2	84.1
4	43.8	119.6
6	39.5	123.3
8	37.9	81.3
10	30.3	47.2

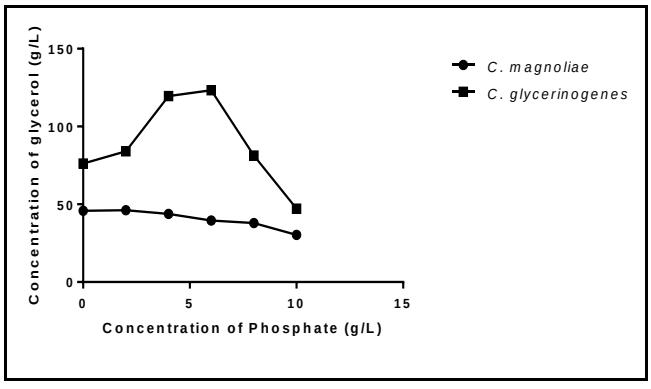

Fig. 2 Effect of initial concentration of phosphate on the production of glycerol in the medium by *C.magnoliae*and *C. glycerinogenes*based on the amount of consumed glucose.

It was found that phosphate is also an important factor in determining the glycerol productivity. A concentration of phosphate between 0 and 2 g/l increases the glycerol yield. Beyond 2 g/l of phosphate the yield of glycerol decreases gradually for *C. magnoliae* simultaneously the glycerol concentration for *C. glycerinogenes decreases* beyond 6 g/l of phosphate.

Effect of Temperature on Glycerol Production

Table 3 Effect of temperature on glycerol productivity

Temperature (^{o}C)	Glycerol for *C. magnoliae* (g/L)	Glycerol for *C. glycerinogenes* (g/L)
26	68.6	79.6
28	73.2	101.1
30	77.3	114.2
32	74.1	130.4
34	70.5	125.7

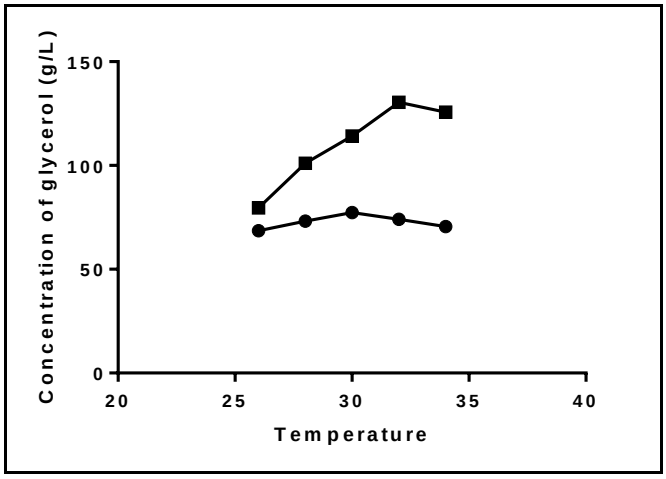

Fig. 3 The temperature significantly affected the production of glycerol by ● *C.magnoliae*and ■ *C.glycerinogenes* based on the amount of glucose consumed.

Using shake-flask culture, the optimum temperature was determined for glycerol production. The concentration of glycerol varies with temperature from 26^{o}C to 34 ^{o}C. The yield of glycerol increases till 30 ^{o}Cfor *C.magnoliae*and beyond this temperature the yield decreases. Similarly for *C.glycerinogene sconce*-tration of glycerol decreases beyond 32 ^{o}C.

Effect of pH on Glycerol Production

Table 4 Effect of pH on glycerol productivity

pH	Glycerol for *C. magnoliae* (g/L)	Glycerol for *C. glycerinogenes* (g/L)
3.0	42.5	113.4
3.5	48.3	117.5
4.0	60.3	120.2
4.5	75.9	128.9
5.0	80.1	134.7
5.5	76.0	130.5
6.0	66.2	127.1
6.5	54.7	112.2
7.0	42.8	103.5

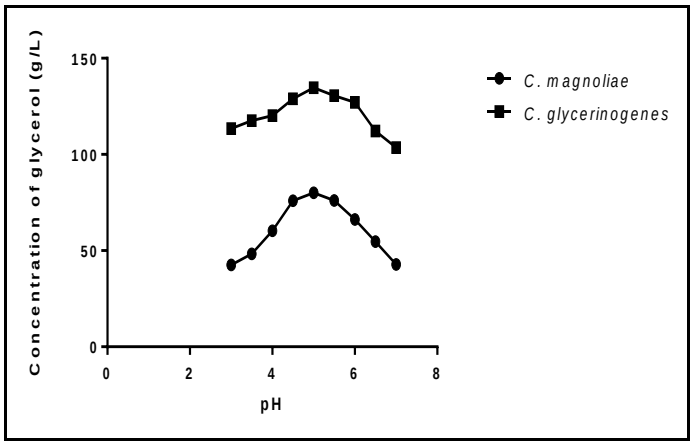

Fig. 4 Effect of initial pH of medium on production of glycerol by *C. magnoliae*and *C. glycerinogenes* ■ based on the amount of glucose consumed.

Batch experiments were done to determine the effect of pH on glycerol production from *C.magnoliae*and *C.glycerinogenes*. At acidic pH the yield was found to be low. Between the pH 4 and 6, the production of glycerol was not significantly affected. At pH 5 there was a significant increase in the growth rate and glycerol production. Finally the optimum pH was found to be 5 for both the organisms.

Discussion

Glycerol yield by microbial fermentation of glucose using *S. cerevisiae*is less than 50%(w/w). But newly discovered *C. magnoliae* andosmotolerent yeast *C. glycerinogenes*produced glycerol in higher concentration compared with *S. cerevisiae.C.glycerimogenes*converts up to 64.5% (w/w) of the available glucose into glycerol under fermentation conditions, with glycerol accumulating up to137 g/l in broth. Whereas *C.magnoliae*converts only 55% (w/w) of the available glucose into glycerol.

Many parameters such as temperature, pH, phosphate and glucose were found to affect the glycerol productivity by *C. magnoliae*and*C. glycerinogenes.*The optimum concentration of glucose for *C. magnoliae*was found to be 160 g/L and for *C. glycerinogenes*was found to be 210 g/L, beyond these concentration range the yield of glycerol decreases. Concentration of phosphate is the important factor for glycerol production. If the concentration exceed the optimum level the cellsgrows slowly or quickly which tends to decrease the glycerol yield.2 g/L of phosphate was estimated as optimum concentration for glycerol production by *C. magnoliae*and similarly for *C. glycerinogenes,*maximum glycerol was obtained at 6 g/L of phosphate[11,12]. 28^{o}C – 35^{o}C of temperature favors the good growth of cells and better glycerol production. Thus the optimum temperatures were declared to be 30^{o}Cfor *C. magnoliae* and 32^{o}C for *C. glycerinogenes.*For glycerol production by osmotolerant yeasts, the yield of glycerol increases with glucose concentration when raised to the optimum concentration, after which production decreases[13].Decreasing thewater activity by increasing the concentration of glucose or salt can also increase the extracellular glycerol concentration greatly in a variety of yeasts[14].

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