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Optimization of Ethanol Production from Pineapple Peel by *Saccharomyces cerevisiae*

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Abstract

The biofuel require growing day by day due to availability of chief renewable sources and easy of production through agro wastes. There are numerous factors that change ethanol production through yeast strain. The major purpose of this work is optimization of procedure for ethanol production by allowing for all the factors. Various optimization conditions such as sugar concentration, temperature, metal ions, pH and immobilization process are used. Through diverse experiments was found temperature 30°C, pH between 5.0 - 6.0, reducing sugar concentration 15-20%, pH were most favorable for highest yield by using *Saccharomyces cerevisiae*. The ethanol production ability of yeast was found 11.2% at pH 6.0, media at 30°C temperature with 15% preliminary sugar concentration in shaky state (120 rpm).Pilot level of ethanol production was found 13.2% at 48 hours. Immobilized cells also were showed more ethanol than free cells and effects of metal salts like magnesium, potassium, boron and chromium were reported on ethanol production. Potassium, Magnesium was showed greatest effect on ethanol production.

Keywords: Biofuel, Immobilization, Metal salts.

1. Introduction

The world progress not even industrial but also technological humanity along with the economically and environmentally implication, like depleting oil reserves as well as global warming have been motivating worldwide attention to developing renewable source, those could be used for biofuel manufacturing as a feedstock. The idiom of biofuel is accredited to substitute fuels. That is derived through organic material like crop residues, waste biomass and energy crops. **(Krugman P, 2008)** In current study, fruits wastes like pineapple peel, these are rich source of cellulosic feedstocks for fermentation of ethanol. These source are also do not interfere by food security. **(Wang, F.S et al., 2000)** Ethanol is a best alternative choice to solve the troubles linked with the expanding energy demands overall world. A great advantage of biofuels which is based on biomass significantly decreases the green house gas secretion. **(Perlack et al., 2005)** Ethanol is an oxygenated fuel along with high octane rate. It's also gives greater performance for the reason that it is run ignition engines at advanced compression rate. **(Wheals et al., 1999)** Ethanol blended with gasoline significantly can be reduce petroleum utilization. Ethanol be capable of a safest alternative to this ordinary additive into gasoline. **(Wang and Sheu 2000)**. In india pineapple is most significant fruit of lignocellulose material for ethanol construction. It has been accepted and exploring worldwide as its accessibility. In india *A. cosmosus* totally produce 1527.93×10^3 tons annually in 15 major states. **(Nishio, N et al., 1980)**

Currently discussed that *Mucor indicus* yeast in the fungus form showed quicker ethanol construction, Their typical efficiency of production 0.90 g/l h as of fructose glucose and sucrose and the filamentous structure through a standard productivity of 0.33 g/l h. **(Sharifia, M. et al., 2008)**

The purpose of this study to development an economically and environmentally friendly bioprocess to generate cellulosic ethanol from pineapple peels. To accomplish this aim through optimizing the process to achieve higher ethanol yield.

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2. Materials and Methodology

2.1 Materials

A.cosmosus (Pineapple) peels were collected from fruit juice vendors of Hyderabad, Telangana, India. Peels were dried in oven for 4–5 days. After drying peels were made fine powder into 100 mesh (0.15 mm) by using of laboratory blender and preserved at 4°C in sealed bag. *Saccharomyces cerevisiae* (MTCC 171) procured from MTCC, Chandigarh.

2.2 Optimization

The fermentation procedure carried out by yeast. Which is vary with different factors like temperature , pH, substrate concentration, inoculum size, metals, N-source and immobilization. Therefore, it's necessary to optimize the condition of fermentation for yeast cells. Different factors were reported distressing production of ethanol from fruit peels.

2.2.1. Effect of temperature

Temperature is a most important for ethanol production, while alcoholic fermentation rate increases along with temperature increased. To optimize temperature fermentation was carried out at 25, 30, 35 and 40°C. For fermentation Fruit was diluted 20% sugars and phosphorus and nitrogen supplement were used. (Morimura, S. et al., 1997)

2.2.2 Effect of pH

The pH were adjusted 5.0, 6.0, 7.0 and 8.0 of the solution for fermentation using fruit peel sample together 20% sugar concentration as well as temperature of 29 ± 2°C. Low pH of the solution inhibits the yeast reproduction

2.2.3 Effect of Sugar concentration

In ethanol fermentation, study of the effect of sugar concentration, production media was prepared to various sugar concentration 15, 20, 25, and 30 percent with H_2O. Inoculum of yeast which is 24 hours old was added at 20 percent in medium. Samples were removed after each 12-hour interval sample were removed and expected for remaining sugars (Miller, Gail Lorenz, 1959) and content of ethanol in the media. (Arthur, C. et al., 1968)

2.2.4 Effect of Metal

To study the effect of metals, in fermentation media was added four metal salts as a source of metal ion. These metal are magnesium chloride Potassium dichromcate, boric acid and copper sulphate.

2.2.5 Effect of Immobilization

Immobilization showed important effect on end product ethanol. Immobilized cell rise ethanol yield. Process were performed at optimum pH 5.5, temperature 30°C, & 6% of reducing sugar. (Mariam, I. et al., 2009)

3. Results and Discussion

3.1 Effect of temperature on ethanol yield

To know optimum temperature the solutions with 20% sugar concentration were kept at 25, 30, 35 and 40°C. At 25°C in 48 hours, a low ethanol yield of 5.8% was observed. At 30°C ethanol yield was maximum to be 13% (Table 1). On the other hand rising the temperature above to 30°C the growth and concentration of alcohol decreased. Optimum temperature for ethanol production 30°C was selected.

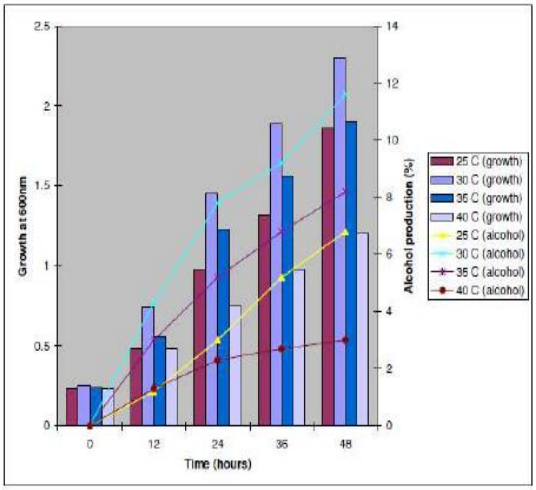

Fig 1: Effect of temperature on alcohol yield

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Table 1: Effect of temperature on Ethanol yield

Time (Hrs)	Growth (in O.D.)				Alcohol (In Percent)			
	25° C	30° C	35° C	40° C	25° C	30° C	35 °C	40 °C
0	0.22	0.26	0.23	0.24	0	0	0	0
12	0.46	0.78	0.50	0.47	1.3	4.0	2.8	1.1
24	0.95	1.55	0.95	0.72	3.1	8.0	4.9	2.1
36	1.31	1.90	1.5	1.45	5.0	9.4	6.7	2.6
48	1.85	2.4	1.8	1.89	5.8	13.2	7.9	2.9

3.2 Effect of pH on ethanol yield

20 % Primary sugar concentration as well as 30°C optimum temperature was chosen for additional studies of pH treatments 5, 6, 7 and 8. At pH 5, low ethanol yield was 7% observed. At pH 6, highest ethanol content 11% was observed here maximum ethanol production was noticed Shown in table 2. When increased pH from 4.0-5.0 increase in alcohol content, also increase productivity and efficiency. **(Yadav et al., 1997)** The pH 6 based on fermentation competence was chosen for additional experimentation.

Table 2: Effect of pH on ethanol yield

Time (Hrs)	**Growth (in O.D.)**				**Alcohol (In Percent)**			
	pH 5	**pH 6**	**pH 7**	**pH 8**	**pH 5**	**pH 6**	**pH 7**	**pH 8**
0	0.21	0.22	0.25	0.21	0	0	0	0
12	0.47	0.85	0.58	0.78	1.9	4.1	2.6	2.2
24	0.97	1.57	1.2	1.5	3.5	8.1	4.4	5.9
36	1.33	1.96	1.6	2.0	4.5	9.3	7.0	7.9
48	1.45	2.46	2.14	2.45	7.7	11.2	8.9	8.9

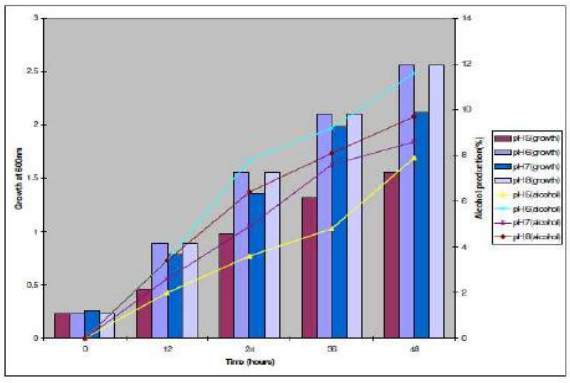

Fig 1: Effect of pH on alcohol yield

3.3 Effect of Reducing Sugar

Ethanol production was increased slightly while primary reducing sugar concentration increasing from 25 to 30% but ethanol production reduce at 9% and 10%. Therefore within 15 to 20% reducing sugar is optimum percentage for all ethanol production (Table 3). So that it was found 15 to 17% of reducing sugar was observed 12% maximum ethanol content after 60 hours.

Table 3: Effect on Reducing Sugar concentration

Time (Hours)	**Growth (in O.D.)**				**Alcohol (In Percent)**			
	15 %	**20 %**	**25 %**	**30 %**	**15 %**	**20 %**	**25 %**	**30 %**
0	0.21	0.25	0.22	0.20	0	0	0	0
12	0.47	0.80	0.48	0.39	1.40	4.5	2.6	1.3
24	1.67	1.78	0.86	0.70	3.45	9.5	4.2	1.8
36	1.96	2.1	1.22	1.39	5.56	9.7	6.5	2.2
48	2.4	2.5	1.31	1.2	7.30	12.2	7.2	2.4

3.4 Effect of Metal

To examine of metals effect on ethanol manufacture by the yeast. Potassium dichromcate Salt was showed 10% highest effect, magnesium chloride was showed 6% slight effective effect but boric acid and copper sulphate were showed inhibitory effect on ethanol production. Result shown on table 4.

Table 4: Effect of Metals on ethanol Yield

Time (Hrs)	**Growth (in O.D.)**				**Alcohol (In Percent)**			
	$MgCl_2$	**$K_2Cr_2O_7$**	**H_3BO_3**	**$CuSO_4$**	**$MgCl_2$**	**$K_2Cr_2O_7$**	**H_3BO_3**	**$CuSO_4$**
0	0.20	0.29	0.19	0.17	0	0	0	0
12	0.44	0.89	0.30	0.20	1.44	4.2	1.22	1.31
24	1.20	1.59	0.45	0.30	4.45	6.7	1.56	1.85
36	1.34	2.4	0.55	0.56	4.56	8.7	1.78	2.29
48	1.89	2.5	0.78	0.69	5.99	10.2	1.88	2.42

3.5 Effect of Immobilization

Immobilization showed important effect on ethanol content. At optimum temperature 30°C, reducing sugar concentration 6% and pH 5.5 originally free cells produced 12.2% (v/v) ethanol yield following 60 hours. At optimum condition immobilized cells produced 14.41% (v/v). (Table 5)

Table 5 : Effect of Immobilization on ethanol yield

Time (Hours)	Growth (in O.D.)		Alcohol (In Percent)	
	Free Cells	Immobilized cells	Free Cells	Immobilized cells
0	0.19	0.17	0	0
12	0.45	0.56	1.8	2.5
24	0.78	0.98	4.6	5.3
36	1.56	1.87	8.9	9.89
48	1.89	2.4	12.2	14.41

4. Conclusions

Thefermentation of pineapple peel using *Saccharomyces cerevisiae* strain under various conditions, sugar concentration 15-20%, temperature at 30°C as well as pH 6.0 are optimum condition, showed 10-12% yield. Ethanol production through free and immobilized cells at 12- 48 hours in shaky condition. Ethanol production reduce after 48 hours because of substrate limitations. Immobilized cells are better than free cells for ethanol production. A few metals like magnesium chloride and Potassium dichromate have showed high effect on ethanol production. This yeast strain could be showed ability for ethanol production from pineapple peels in commercial level. However, through genetic manipulation and radiation ethanol production can be increase.

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