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Bio-Ethanol Production from Unrefined Sugar by Fermentation Using the Strain *Saccharomyces cerevisiae*

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In this study, *Saccharomyces cerevisiae* is used for bio-ethanol production from unrefined sugar under anaerobic condition. The parameters that influence the production of bio-ethanol from unrefined sugar are optimized. The optimal values of the parameters such as sugar concentration, enzyme concentration, temperature and pH are found to be 300 gm/L, 2 gm/L, 38 ºC and 4 respectively. Under this optimum operating conditions the maximum yield of bio-ethanol is 63 %. The rate of formation of bio-ethanol is found to be well fitted with Michaelis-Menten equation and the values of V_{max} and K_m are found to be 0.71 mol/L sec and 81.63 mol/L, respectively.

Key Words: Bio-ethanol, Fermentation, Optimization, Unrefined sugar, *Saccharomyces cerevisiae*.

INTRODUCTION

Energy availability, supply and use play a central role in the way societies organize themselves from individual welfare to social and industrial development. By extension, energy accessibility and cost is a determining factor for the economical, political and social interrelations among nations. Considering energy sources, human society has dramatically increased the use of fossil fuels in the past 50 years in a way that the most successful economies are large consumers of oil[1-3]. However, geopolitical factors related to security of oil supply, high oil prices and serious environmental concerns, prompted by global warming have led to a push towards decreased consumption. Indeed, the world's strongest economies are deeply committed to the development of technologies aiming at the use of renewable sources of energy. Within this agenda, the substitution of liquid fuel gasoline by renewable bio-ethanol[4-11] is of foremost importance.

In this present research renewable bio-ethanol is fermented from the unrefined sugar using the strain *Saccharomyces cerevisiae*[12,13]. The traditional unrefined sugar made from the sap of the date palm and cane juice without separation of the molasses and crystals was taken as the feedstock. An attempt has been made to optimize the variables[14-16], which affect the bio-ethanol production from unrefined sugar and the experimental results are compared with the available reaction kinetics[17].

EXPERIMENTAL

A known quantity of unrefined sugar is made up to 1 L using water. This is taken in the fermentation flask with the yeast *Saccharomyces cerevisiae* and maintained at a pH range of 6-7 at 29 ºC under anaerobic condition. Fermentation was completed within 4-5 days. The strain converted sugar into bio-ethanol with the evolution of CO_2. The fermented sample was collected for every 12 h interval and the bio-ethanol concentration was determined by gas chromatography. The procedure was adopted to optimize the parameters such as pH, temperature, sugar concentration and yeast concentration.

Identification of bio-ethanol: About 5 to 10 mL of fermented sample was taken and a pinch of potassium dichromate and a few drops of H_2SO_4 were added. The colour of the sample turns to pink which indicates the presence of bio-ethanol.

Determination of bio-ethanol concentration and pH: Bio-ethanol concentrations were determined by gas chromatography, using a CG-3537D gas chromatograph manufactured by instruments scientifics CG LTDA with a flame ionization detector and a CG-300 integrator. The pH was determined with a B272 pH digital meter, a Micronal make.

RESULTS AND DISCUSSION

Optimization of sugar concentration: Different concentrations of unrefined sugar such as 50, 100, 200, 300 and 400 g/L are made and fermented using *Saccharomyces cerevisiae* at a pH range of 6-7 at 29 ºC. The sample was collected for every 12 h interval and transferred to distillation chamber (simple distillation) for purification and then concentration of bio-ethanol was estimated using gas chromatography. Table-1 (Fig. 1) shows that the concentration of bio-ethanol increases with the increase in sugar concentration and reaches maximum bio-ethanol production at sugar concentration of 300 g/L and further increase in sugar concentration inhibit the bio-ethanol productivity.

TABLE-1
OPTIMIZATION OF SUGAR CONCENTRATION

Sample no.	Sugar conc. (g/L)	Yeast conc. (g/L)	Bio-ethanol concentration (%)						
			12 h	24 h	36 h	48 h	60 h	72 h	86 h
1	50	2	3	10	13	18	20	19	18
2	100	2	6	18	24	27	29	29	26
3	200	2	8	19	30	42	46	49	49
4	**300**	**2**	**11**	**23**	**35**	**46**	**51**	**53**	**53**
5	400	2	15	22	24	25	25	23	19

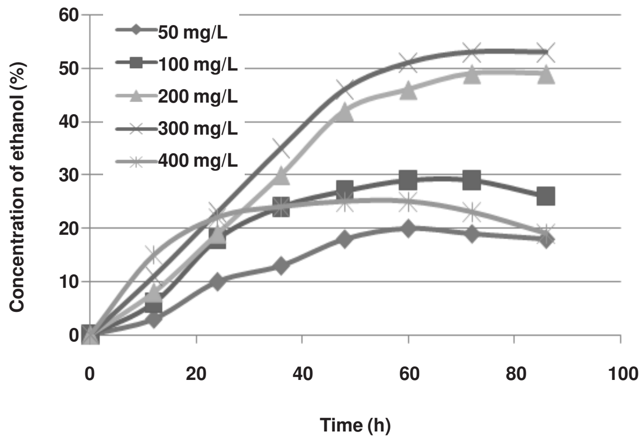

Fig. 1. Optimization of sugar concentration

Optimization of yeast concentration: The optimized quantity of unrefined sugar (300 g/L) was taken and the pH and temperature were maintained at 6-7 range and 29 ºC. Fermentation was tried with different concentrations of yeast like 1, 2, 4 and 8 g in anaerobic condition. The fermentation solution was collected for every 12 h interval and transferred to distillation chamber (simple distillation) for purification. The concentration of bio-ethanol was estimated. Table-2 (Fig. 2) shows that as the concentration of yeast increases, the yield of bio-ethanol increases. Above 2 g/L of yeast concentration the bio-ethanol yield decreases. The optimized yeast concentration is 2 g/L.

TABLE-2
OPTIMIZATION OF YEAST CONCENTRATION

Sample no.	Sugar conc. (g/L)	Yeast conc. (g/L)	Bio-ethanol concentration (%)						
			12 h	24 h	36 h	48 h	60 h	72 h	86 h
1	300	1	5	10	18	29	33	39	38
2	**300**	**2**	**11**	**23**	**35**	**46**	**51**	**53**	**53**
3	300	4	12	20	29	32	39	44	42
4	300	8	15	21	29	32	36	33	28

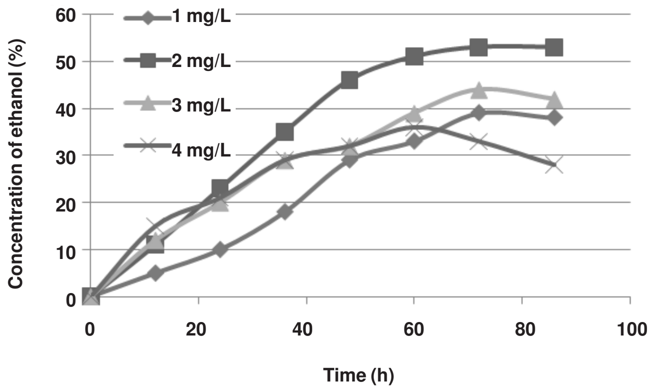

Fig. 2. Optimization of yeast concentration

Effect of temperature: The sample for fermentation prepared with optimized sugar concentration (300 g/L) and yeast concentration (2 g/L) was fermented at different temperatures like 25, 30, 38 and 45 ºC in anaerobic condition at pH range 6-7. The fermented solution was collected for every 12 h interval transferred to distillation chamber (simple distillation) for purification. The concentration of bio-ethanol was estimated by gas chromatography. Table-3 (Fig. 3) shows bio-ethanol production increases with the increase in temperature and reaches a maximum value of 38 ºC. Further increase in temperature reduces bio-ethanol production and it is mainly due to denature of yeast cells.

TABLE-3
EFFECT OF TEMPERATURE

Sample no.	Sugar conc. (g/L)	Yeast conc. (g/L)	Temp ºC	Bio-ethanol concentration (%)					
				12 h	24 h	36 h	48 h	60 h	72 h
1	300	2	25	9	20	28	33	36	39
2	300	2	30	11	23	35	46	53	53
3	**300**	**2**	**38**	**12**	**25**	**37**	**50**	**59**	**63**
4	300	2	45	2	5	7	7	6	4

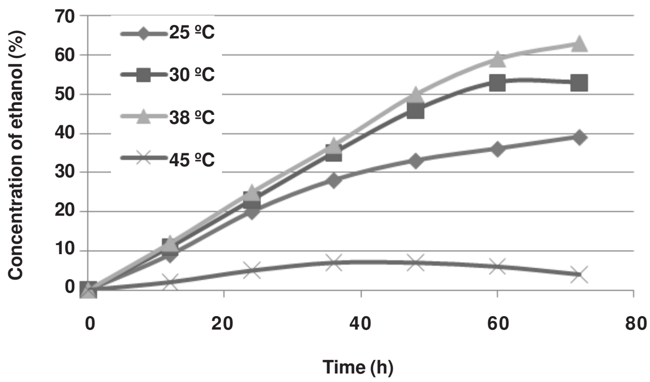

Fig. 3. Effect of temperature

Effect of pH: The sample was fermented at different pH values between 1 and 8 using optimized yeast concentration (2 g/L) at optimum temperature under anaerobic condition. The fermented solution is collected for every 12 h interval and transferred to distillation chamber (simple distillation) for purification. The concentration of bio-ethanol was measured using gas chromatography. Table-4 (Fig. 4) shows that bio-ethanol concentration gradually increases along with the increase in pH and reaches maximum at 4 pH and beyond this bio-ethanol production decreases.

TABLE-4
EFFECT OF pH

Sample no.	Sugar conc. (g/L)	Yeast conc. (g/L)	pH	Bio-ethanol concentration (%)			
				24 h	48 h	72 h	96 h
1	300	2	1	0	0	0	0
2	300	2	2	0	10	11	11
3	300	2	3	5	12	24	36
4	**300**	**2**	**4**	**19**	**29**	**53**	**59**
5	300	2	5	18	26	50	53
6	300	2	6	3	4	15	20
7	300	2	7	0	0	8	10
8	300	2	8	0	0	3	5

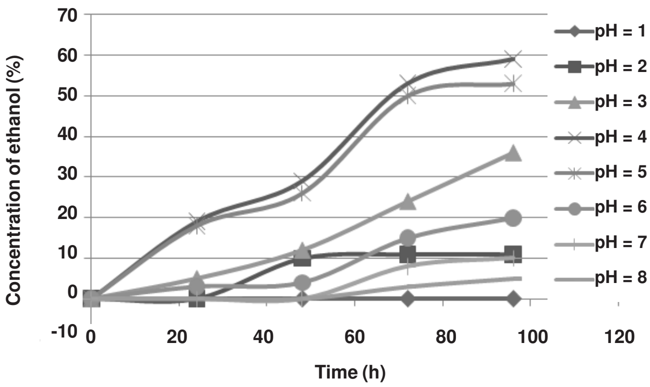

Fig. 4. Effect of pH

Productivity of bio-ethanol from unrefined sugar: The productivity of bio-ethanol is shown in Table-5 and Fig. 5. As the fermentation period increases the productivity increases and reaches a maximum of 63 % at 72 h at optimized conditions.

TABLE-5
PRODUCTIVITY OF BIO-ETHANOL

Unrefined sugar concentration (gm/L)						300	
Yeast concentration (gm/L)						2	
pH						4	
Temperature (ºC)						38	
Time (h)	12	24	36	48	60	72	86
Bio-ethanol conc. (gm/L)	12.34	30.84	53.97	77.1	83.27	90.98	90.98

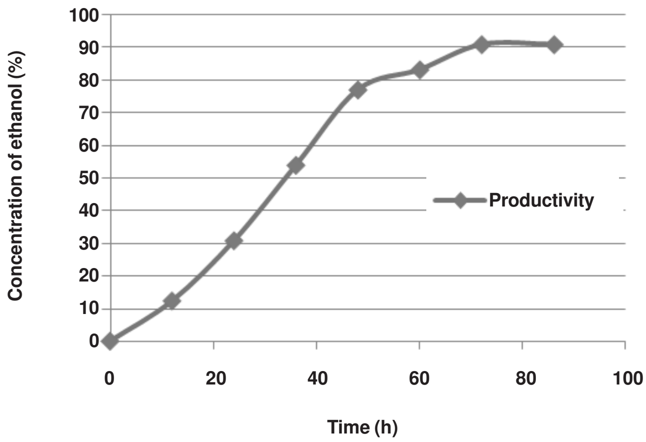

Fig. 5. Productivity of bio-ethanol

The rate of formation of bio-ethanol was compared with Michaelis-Menton equation and found to be good fit and the rate constant K_m and V_{max} are found to be 81.63 mol/L and 0.71 mol/L sec, respectively from Fig. 6.

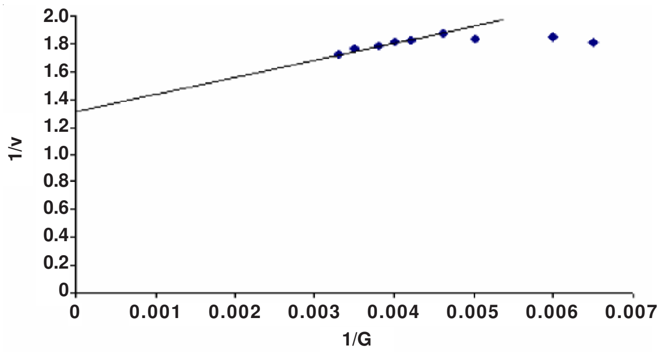

Fig. 6. Plot 1/G *vs*. 1/v

Conclusion

In the fermentation of unrefined sugar to bio-ethanol under anaerobic condition the parameters like sugar concentration, temperature, pH and yeast concentration were optimized for maximum yield and found to be 300 g/L, 38 ºC, 4, 2 g/L respectively. The results are compared with Michaelis-Menton equation and the values of V_{max} and K_m are found to be 0.71 mol/L sec and 81.63 mol/L, respectively.

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