Pakistan J. Sci. Ind. Res., Vol. 31, No. 11, November 1988

BATCH ALCOHOLIC FERMENTATION OF MOLASSES: EFFECTS OF pH, TEMPERATURE AND SUGAR CONCENTRATION

R.A. Oderinde and K.O. Esuoso

Department of Chemistry, University of Ibadan, Ibadan

(Received February 10, 1988; revised November 28, 1988)

The effects of pH, sugar concentration and temperature on the alcoholic fermentation of Nigerian molasses were studied. The various cultures were inoculated with yeast inoculum having 5.2 – 6.1 cells per ml. When pH values were in the region of 1.0 and 3.0, ethyl alcohol concentrations were below 1.0% v/v, whereas maximum ethyl alcohol concentration was produced between 4.0 and 5.0. The concentration of ethyl alcohol ranged from 7.9 to 8.7% v/v. However at a higher pH value of 6.0, the concentration of ethyl alcohol recorded was 7.8% v/v. Optimum sugar concentration ranged from 14.0 to 1.70% w/v, while that of ethyl alcohol lies between 7.32 to 9.06% v/v. The optimum temperature which gave the maximum ethyl alcohol concentration (8.2% v/v) was 28.0^{o} . Nigerian cane molasses is quite suitable for industrial production of ethyl alcohol.

Key words: Sugar, Fermentation, Molasses.

INTRODUCTION

Due to the current interest in alcohol production from renewable sources, attempts have been made to optimize alcoholic fermentation various carbohydrate substrates by batch process. While certain substrates like molasses, glucose syrup, apple pomace, cheese whey and grape juice [1-3] and cellulose containing substances [9] require extensive hydrolytic treatments coupled with the problem of accumulation of toxic products. Hence the factors which affect alcoholic fermentation may invariably differ from one substrate to the other. The major problem associated with fermentation routes for ethyl alcohol is that the reaction has to be carried out in dilute solutions with a consequence of the yield of ethanol becoming too low to recover economically [10]. In alcoholic fermentation of molasses, the optimum sugar concentration to which molasses should be diluted depends on the yeast strain tolerance, adaptation to osmotic effects of the broth, other assimilable compounds in the medium, concentration of ash and toxicity of the medium [11]. In addition it depends on the toxicity effects of trace metals [12]. Other factors like pH, yeast concentration, aeration, nutrient supplementation and temperature also exerts profound influence on the rate of fermentation and the ethyl alcohol content. It is however difficult to investigate the quantitive effects of all these factors together in a single set of experiment. This article describes the effect's of pH, sugar concentration and temperature on the yield of ethyl alcohol from sugar cane molasses.

MATERIALS AND METHODS

Organism.The strain of yeast *Saccharomyces cerevisiae* was obtained from Department of Botany and microbiology of the University of Ibadan. The culture was maintained on malt extract. agar medium consisting of: (g/l) malt extract, 30.0; mycological peptone, 5.0; agar, 15.0. inoculum preparations.

The yeast was grown in malt extract broth medium. In the development of inoculum the medium was sterelized at 121^{o} for 15 minutes.

Fermentation technique. Cane molasses obtained from Bacita sugar factory in Kwara State was diluted with water to various sugar concentrations and autoclaved at 103.4 KNm^{-2} for 15 min. Ammonium sulphate 1.0 g/l was added to the flask as additional nutritional supplement for the yeast. Fermentation was carried out in a 500ml conical flask. Containing 200ml sample of the mash . It was seeded asceptically a yeast population of 5.2 - 6.1 x 10^{6} cells/ml and fermented for 48 hours.

Analytical methods. Sugar was determined by both the dinitrosalicylic acid method [13] and Lane and Eyons constant volume titrimetric method [14]. Zinc ferrocyanide was used to clarify and molasses.

Alcohol content. Ethyl alcohol content in the fermented broth was determined by the Dichromate oxidation method [15].

Yeast count. Yeast cell number was determined using hemocytometer slides.

RESULTS AND DISCUSSION

The effects of pH and sugar concentration are shown in Fig. 1, while the fermentation patterns with changes in pH sugar concentration and temperature are presented in Tables 1, 2 and 3 respectively.

At pH values between 1.0 and 3.0, the concentration of ethyl alcohol in the medium is low (below 1.0 v/v),

Table 1. Effect of pH on the fermentation pattern of sugar cane molasses

	Before fermentation					After fermentation		
Sample	pH of the medium	Reducing sugars (% w/v)	Sucrose (% w/v)	Total sugars (% w/v)	Temperature of the medium (°C)	pH of the medium	Total sugars (% w/v)	Temperature of the medium (°C)
1	1..00	5.39	9.51	14.90	29	1.50	13.72	30
2	1.50	5.84	8.77	14.61	29	2.00	12.91	31
3	2.00	5.82	9.03	14.85	29	2.35	12.60	31
4	2.50	7.09	7.89	14.98	29	2.85	10.30	31
5	3.00	7.21	7.29	14.50	29	3.20	7.17	32
6	3.50	6.20	8.32	14.52	29	3.60	5.23	32
7	4.00	5.22	9.20	14.42	29	4.30	4.44	32
8	4.50	5.66	8.94	14.60	29	4.55	1.15	32
9	5.00	5.96	8.89	14.85	29	4.65	1.11	32
10	6.00	5.87	8.93	14.80	29	4.50	2.22	31

Table 2. Effect of sugar concentration of mash on the fermentation pattern of sugar cane molasses

	Before fermentation			After fermentation		
Sample	pH of medium	Reducing sugars % w/v	Sucrose % w/v	Total sugars % w/v	pH of medium	Total sugars % w/v
1	4.50	11.96	21.06	33.02	4.55	17.05
2	4.50	10.73	16.09	26.80	4.60	3.64
3	4.40	10.36	16.04	26.40	4.50	3.82
4	4.60	10.97	12.19	23.16	4.55	3.02
5	4.72	6.96	10.40	17.36	4.60	4.21
6	4.50	5.57	9.06	14.63	4.55	1.34
7	4.45	5.09	8.06	13.15	4.40	1.56
8	4.50	5.08	7.71	12.79	4.55	2.34
9	4.50	4.71	8.31	13.02	4.54	1.96
10	4.65	4.40	6.51	10.91	4.56	5.27
11	4.55	3.96	6.21	10.17	4.60	5.21
12	4.55	3.72	5.87	9.59	4.60	5.46
13	4.50	3.77	5.07	8.84	4.52	7.56

Table 3. Effect of temperature on the fermentation pattern of sugar cane molasses.

	Before fermentation					After fermentation		
Sample	Temperature of medium °C	pH of medium	Reducing sugars % w/v	Sucrose % w/v	Total sugars % w/v	pH of medium	Total sugars % w/v	Concentration of ethyl alcohol % v/v
1	8.0	4.50	5.42	9.54	14.96	4.55	7.29	1.45
2	8.0	4.55	5.45	9.60	15.05	4.62	8.19	1.24
3	28.0	4.45	6.24	8.55	14.79	4.50	3.65	8.20
4	28.0	4.50	5.54	9.38	14.92	4.54	3.58	7.83
5	50.0	4.50	5.48	9.20	14.68	4.55	6.40	3.53
6	50.0	4.60	5.70	9.02	14.72	4.72	6.96	3.48

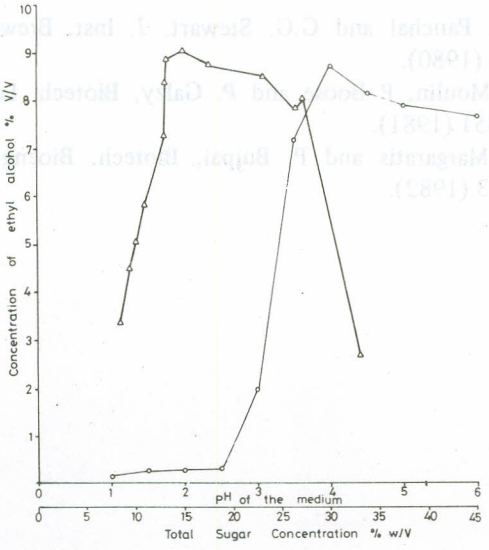

Fig. 1. –■–■– Effect of pH on the production of ethyl alcohol from cane molasses by *Saccharomyces cerevisiae.*

–▲–▲– Effect of sugar concentration on the production of ethyl alcohol from cane molasses by *Saccharomyces cerevisiae.*

while residual sugars were still high, which implies little fermentative activity of the yeast at those pH values. However optimum concentration of ethyl alcohol was found at pH values in the region of 7.9 and 8.7%. Similar results were recorded by du Perez *et al* during fermentation using *Pichia Stiptis* and *Candida shehatae* [16]. Tolerance of yeasts to pH, changes has also been reported [17, 18]. In this study the optimum pH values for maximum growth of yeast ranged from 4.0 to 5.5.

The optimum sugar concentration lies within the range of 14.0 to 17.0% v/v. However at sugar concentrations greater than 17.0% w/v ethyl alcohol concentration decreased gradually reaching a minimum of 2.70% v/v with 33.0% w/v total sugars. Likewise when the sugar concentrations was lower than 14.0% w/v, ethyl alcohol concentration also reduced gradually to a minimum of 3.4% v/v with 9.0% w/v total sugars. The lower yield of ethyl alcohol at higher sugar concentrations may be due to the osmotic effects on the yield and the possibility of the production of glycerides aldehydes and higher molecular weight alcohols. [11, 19, 20]. In addition, it can be due to ethanol inhibition, since at high sugar concentrations, more ethanol is produced which can stop the fermentation at an early stage [21 - 24]. The decrease in the yield of ethyl alcohol with lower concentration of sugar is due to low cell - mass concentration [25].

Maximum ethyl alcohol concentration was produced at 28 ^{o} while lower temperature 8.0^{o} resulted in a decrease of ethyl alcohol. However higher temperature 50.0^{o}) produced a better yield than those cultures maintained at 8.0^{o} but lower than those at 28.0^{o}.

CONCLUSION

The effect of pH, sugar concentration and temperature on the fermentation of molasses to ethyl alcohol was determined. The optimum pH value is b/w 4.0 and 5.0, temperature between 25.0 and 30.0^{o} and optimum sugar concentration between 14.0 and 17.0% w/v. The fact that alcohol concentration is maximum up to 17.0% v/v implies that molasses medium is not too harzadous to yeast or the yeast strain has tolerance to the adverse effects of the medium. Nigerian cane molasses is quite suitable for industrial alcohol production.

REFERENCES

1. Y.D. Hang, C.Y.Lee and E.E. Woodams, J. Food Sci., **47**, 1851 (1982).
2. V.S. O'leary, R. Green, B.C. Sullivan and V.H. Holsinger, Biotech. Bioeng., **19** 1019 (1977).
3. N. Kosaric, A. Wieczorek and S. Kliza, Biotech. Bioeng., **25**, 1737 (1983).
4. P. Thammarutwasik, Y. Koba and S. Ueda, Biotech. Bioeng., **28**, 1122 (1986).
5. S. Ueda, C.T. Zenin, D.A. Monteiro and Y.K. Park, Biotech. Bioeng., **23**, 291 (1981).
6. A. Margaritis and P. Bajpai, Biotech. Bioeng., **24**, 941 (1982).
7. J.O. Carioca and J.B. Scares, Biotech. Bioeng., **20**, 444 (1978).
8. K. Kim and M.K. Hamdy, Biotech. Bioeng., **28**, 138 (1986).
9. L. Zeruche and R.R. Zall, J. Fd. Sci., **47**, 328 (1981).
10. E. Gabler, R. J. Kalter, R.N. Boisvert, L.P. Walker, R.A. Pellerin and A.M. Rao, In Proceedings of Food and Fuel from Ethanol Production Symposium NRAES Report Cornell University, Ithacq N.Y. (1982), No. 17, 13.
11. J.S. Harrison and J.C. Graham, *Yeasts in Distillery* Practice, In "The Yeasts" (A.H. Rose and J.S. Harrison eds Academic press, London 1970) Vol. 3.
12. R.A. Oderinde, L.C. Ngoka and E.K. Adesogan, Biotech. Bioeng., **28**, 1462 (1986).
13. G.L. Miller, Anal. Chem., **31** (3), 426 (1959).
14. D. Pearson, *Chemical Analysis of Foods* (Churchill Livingstone London, 1982) 8th ed.
15. A. Capinti and D. Wright, J. Assoc. Off. Anal. Chem. **52** (1), 85 (1969).
16. J.C. du Perez, M. Bosch and B.A. Prior, Enzyme Microb. Technol., **8**, 360 (1986).
17. W.D. Maxon and M.J. Johnson, Ind. Eng. Chem., **45**, 2554 (1953).
18. K. Visuri and B.H. Kirsop, J. Ins. Brewing, **76**, 362 (1970).
19. M.Y. Savchuk and P.S. Tsygankov Vyssh, Ucheb,

Zared Pishch Tekhnol, **6**, 60 (1970).,
20. M.Y. Savchuk and B. Tsygankov Kharchova Prom., **6**, 30 (1970).
21. W.D. Gray, J. Bacteriol, **42**, 561 (1941).
22. G.R. Cysewski and C.R. Wilke, Biotech. Bioeng., **20**, 1421 (1978).
23. C.J. Panchal and G.G. Stewart, J. Inst. Brew., **86**, 207 (1980).
24. G. Moulin, P Boose and P. Galzy, Biotech. Letters, **3**, 351 (1981).
25. A. Margaratis and P. Bujpai, Biotech. Bioeng., **24**, 1473 (1982).