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Research Paper

The Bioproduction of Ethanol through Isolation of Some Local Bacteria

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Abstract

The present study describes the ethanol fermentation from sugarcane molasses by locally isolated yeast strain. Ten yeast strains were isolated from soil and cultured in 15% molassess medium. *Saccharomyces cerevisiae* Bio-07 gave maximum productivity (52.0g/L). Fermentation conditions were optimized for maximum production of ethanol. Maximum yield of ethanol (76.8 g/L) was obtained with 15% molasses concentration, 3% inoculum size, pH 4.5 and temperature 30 ° C. Potassium Ferrocyanide (150ppm) was used to control the trace metals present in the molasses medium.

Keywords: Sugarcane Molasses; Ethanol Fermentation; *Saccharomyces Cerevisiae* Bio-07; Optimization

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1. Introduction

Bio-ethanol is an eco-friendly fuel that can be used in unmodified petrol engines [1-2]. Combustion of ethanol results in relatively low emission of volatile organic compounds, carbon monoxide and nitrogen oxides. The emission and toxicity of ethanol are lower than those of fossil fuels such as petroleum, diesel etc. [2]. Molasses is widely used as a raw material for the production of ethanol for economic reasons, and different strains of yeast have been selected for efficient ethanol production [3-5]. Utilization of molasses for the production of ethanol will not only provide value addition to the byproduct fermentation. *Saccharomyces cerevisiae* is the cheapest strain used for bio-ethanol production from sugar molasses. *S. cerevisiae* is capable of very rapid rates of ethanol production under optimal conditions [6]. In the present work, some factors affecting the ethanol productivity of yeast in molasses were optimized.

2. Materials and Methods

2.1 Organism

The cultures of *Saccharomyces cerevisiae* were isolated from soil by pour plate method. Dry powdered yeast was also used. The samples were streaked on nutrient agar medium and incubated at 30°C for 24h. The cultures were screened (Table 1) for ethanol production in sugarcane molasses medium. The best culture was selected, identified and designated as *Saccharomyces cerevisiae* Bio-07. All cultures were stored in refrigerator at 4 °C.

2.2 Fermentation Technique

Batch fermentation was carried out in 250ml conical flasks. Sugarcane molasses was obtained from Pattoki sugar mill. Sugar concentration in sugarcane molasses was 40% (w/v). Sulphuric acid was added to adjust the pH. Inoculum was prepared by inoculating cells from 24h old slant culture into yeast extract agar medium and incubated at 30 °C for 24 hr. inoculum (2%) was added into molasses for ethanol production.

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Table 1. Screening of *Saccharomyces cerevisae* strains for ethanol production in sugarcane molassess medium.

Serial no.	Yeast strains	Ethanol production (g/L) Mean ± S.E
01	Bio-01	49.1± 0.01
02	Bio-02	51.5± 0.03
03	Bio-03	49.1± 0.04
04	Bio-04	51.9± 0.01
05	Bio-05	51.3± 0.02
06	Bio-06	49.7± 0.01
07	Bio-07	52.0± 0.03
08	Bio-08	50.5± 0.05
09	Bio-09	49.5± 0.04
10	Bio-10	49.7± 0.01

2.3 Analytical Method

2.3.1 Sugar estimation

Sugar concentration in sugar cane molasses was estimated using 3, 5-dinitrosalicylic acid and glucose as standard [7].

2.3.2 Ethanol estimation:

The levels of ethanol were measured by gas chromatography with a flame ionization detector [8].

3. Results and Discussion

3.1 Isolation and screening of Saccharomyces cerevisiae

Ten cultures of *Saccharomyces cerevisiae* were isolated from soil and screened for the production of ethanol. The locally isolated *Saccharomyces cerevisiae* Bio-07 gave better (52.0g/L) ethanol production after 48h of inoculation. *Saccharomyces cerevisiae* Bio-07 was selected for further studies.

3.2 Effect of sugar concentration

Molasses concentration was varied (5, 10, 15, and 20 %) to study their effect on ethanol fermentation by *Saccharomyces cerevisiae* (Figure 1). It was observed that ethanol production was maximum (61.5 g/L) after 48h of inoculation when sugar concentration was 15 %. It was observed that with increase in sugar concentration ethanol production decreased. Hexose sugar is the primary reactant in yeast metabolism. Under fermentative condition, the rate of ethanol production is related to the available sugar concentration [9-11]. At very low substrate concentration, the yeast starved and productivity decreases [12]. An important secondary effect of higher sugar content is catabolite repression of the oxidative pathways [13].

3.3 Effect of inoculum size

The size of inoculum in ethanol fermentation is of great importance in completing the fermentation process. Different sizes of inoculum 1-5 %(v/v) were used to inoculate the production flasks. The amount of ethanol produced gradually increased with the increase in the inoculum size. However, it was found that maximum ethanol production (65.0g/L) was achieved at 3.0% (v/v) inoculum. Further increase in inoculum size did not result in the considerable enhancement of ethanol production (Figure 2). This finding is in agreement with other workers (Bajaj *et al*., 2001; Nowak, 2001; Kordowska-Wiater *et al.*, 2001 and Alegre *et al.,* 2003).

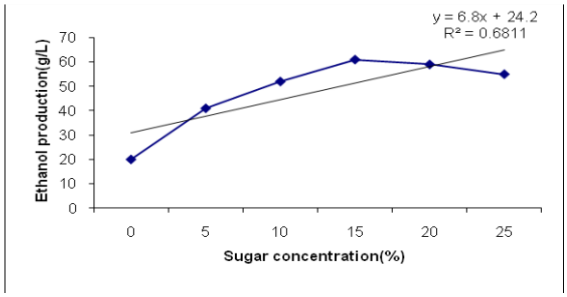

Figure 1. Effect of sugar concentration on ethanol production by *Saccharomyces cerevisiae* Bio-07 using sugarcane molasses. Fermentation conditions: sugar concentration 15%, Incubation period 48h, Temperature 30°C, Inoculum size 2%, pH 4.5

3.4 Effect of initial pH value on ethanol fermentation

Initial pH of the fermentation media was maintained in the range of 2.5 - 6.0. The maximum ethanol production (65g/L) was achieved at pH 4.5. With a further increase in pH ethanol production was decreased (Figure 3). This finding is in consistence with Molisson, 1993 and Hodge, 1953. Control of pH during ethanol fermentation is important for two reasons: 1) the growth of harmful bacteria is retarded by acidic solution. 2) Yeast grows well in acidic conditions [11]. With increase in pH yeast produces acid rather than alcohol. Molasses has naturally alkaline pH and must be acidified prior to fermentation [13-15].

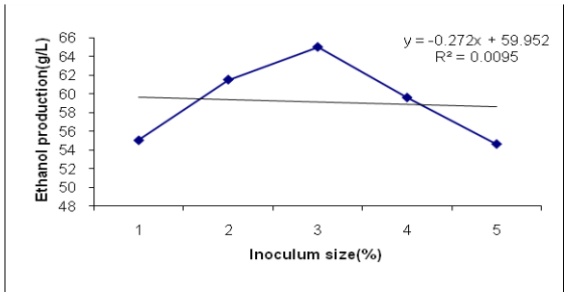

Figure 2. Effect of inoculums size on ethanol production by *Saccharomyces cerevisiae* Bio- 07 from sugarcane molasses. Fermentation conditions: Incubation period 48h, Temperature 30°C, Sugar concentration 15%, pH 4.5

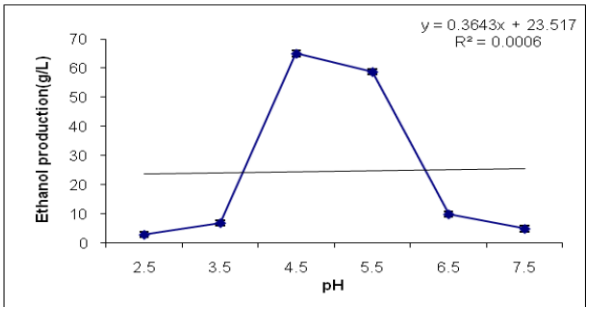

Figure 3. Effect of pH on ethanol production by *Saccharomyces cerevisiae* Bio-07 using sugarcane molasses. Fermentation conditions: Incubation period 48h, Temperature 30°C, sugar concentration 15%, Inoculum size 3%.

3.5 Effect of temperature on ethanol fermentation

Temperature has profound effect on ethanol fermentation. Ethanol production was optimum at 30°C and ethanol production decrease to 0.50g/L at 40°C (Figure 4). This is in agreement with work reported by other workers [16]**.** Temperature between 30-35°C has been usually employed for culturing of yeast and temperature above 30°C has been found inhibitory to ethanol fermentation due to yeast growth inhibition at higer temperatures [17].

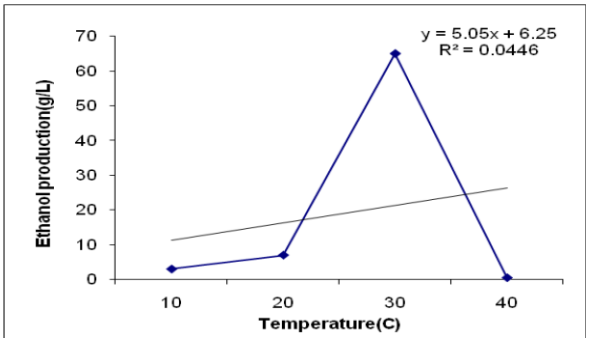

Figure 4. Effect of temperature on ethanol production by *Saccharomyces cerevisiae* Bio- 07 using sugarcane molasses. Fermentation conditions: Incubation period 48h, Inoculum size 3%, Sugar concentration 15%, pH 4.5

3.6 Effect of aeration on ethanol fermentation

Optimum ethanol (65g/L) was working volume of flasks was 800ml in 1000ml conical flask. Further increase in the volume of media caused a decrease in ethanol production (Figure 5). It is important to avoid a high degree of aerobic metabolism which utilizes sugar substrate but produces no ethanol. It has been found, however, that trace amounts of oxygen may greatly stimulate yeast fermentation. Oxygen is required for yeast growth as a building block for the biosynthesis of polyunsaturated fats and lipids required in mitochondria and the plasma membrane [18-19]. Trace amounts of oxygen are adequate and do not promote aerobic metabolism. Typical consequences of oxygen deficiency are restricted growth, reduced yeast viability and slow and incomplete fermentation (Cysewski and Wilke, 1976).

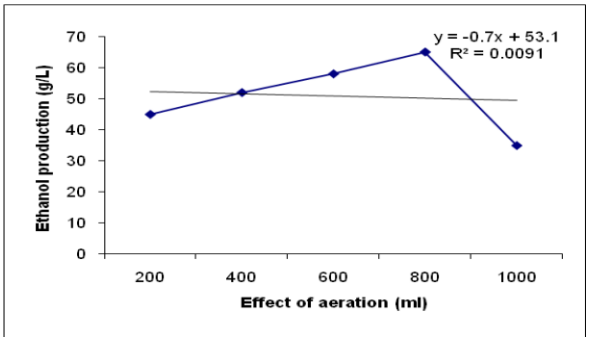

Figure 5. Effect of volume of media on ethanol production by *Saccharomyces cerevisiae* Bio-07 using sugarcane molasses. Fermentation conditions: Incubation period 48h, Temperature 30°C, Sugar concentration 15%, pH 4.5

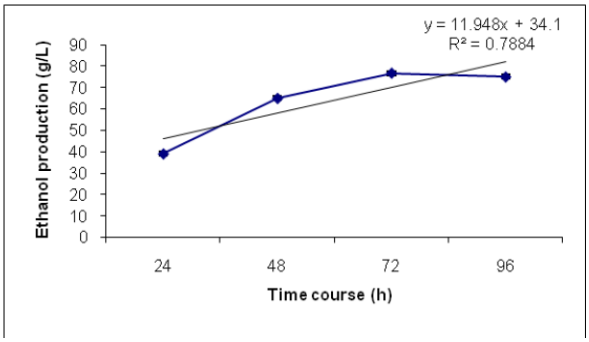

Figure 6. Effect of time course on ethanol fermentation by *Saccharomyces cerevisiae* Bio-07 using sugarcane molasses. Fermentation conditions: Incubation period 48h, Temperature 30°C, Sugar concentration 15%, pH 4.5

3.7 Time Course of ethanol fermentation

The fermentation was carried out at different time period 24, 48, 72 and 96h under optimum conditions. Maximum ethanol production was observed after 72h (76.78 g/L) after inoculation (Figure 6). The ethanol production rate is the product of specific (per cell) productivity and concentration of cells. Initially, the rate of alcohol production is quite low, but as the number of yeast cells increases the overall production rate increases. The effect of reduced sugar concentration and ethanol inhibition becomes important after optimum fermentation time. The fermentation continues at a decreasing rate until 94%of the sugar is utilized. Fermentation time also varies with yeast strains and substrates being used as source of sugars.

Figure 7 shows the analysis of residual sugar concentration and ethanol production during ethanol fermentation from sugarcane molasses by *Saccharomyces cerevisiae* Bio-07. Initial sugar concentration in the molasses was 15% but after

72h sugar concentration was reduced to 6% and ethanol production was 76.78g/L. Further increase in time period resulted in decrease in sugar concentration and ethanol production.

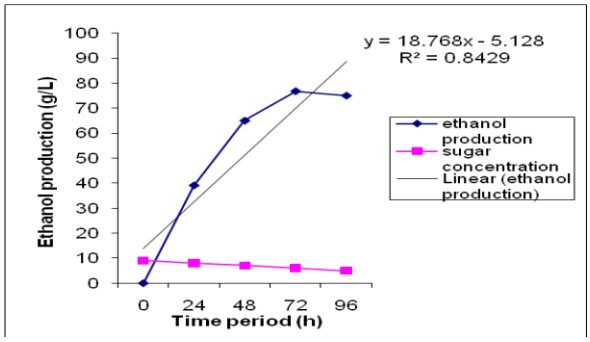

Figure 7. Analysis of residual sugar concentration during ethanol production by *Saccharomyces cerevisiae* Bio-07 using sugarcane molasses. Fermentation conditions: Incubation period 48h, Temperature 30°C, Sugar concentration 15%, pH 4.5

4. Conclusion

It was concluded from the present work that *Saccharomyces cerevisiae* Bio- 07 has great potential for the production of ethanol from sugarcane molasses. The results indicated that the optimization of cultural conditions, such as sugar concentration, inoculum size, pH, temperature, aeration and time of fermentation can further enhance ethanol production.

References

1. Alegre, R.M., Rigo, M. and Jokes. 2003. Ethanol fermentation of a diluted molasses medium by saccharomyces cerevisiae immobilized on chrysotile. *Braz. Arch. Biol. Technol.* 46(4).
2. Atiyeh, H., Duvnjak, Z. 2001. Production of fructose and ethanol from cane molasses using *Saccharomyces cerevisiae* ATCC 36858. *Acta Biotechnologica.* 23(1): 37-48.
3. Bajaj, K.B., Yousef, S., Thakur, L.R. 2001. Selection and characterization of yeasts for desirable fermentation characteristics. *Indian J. Microbiol*. 41(2): 107-110.
4. Beuchat, L. R. 1983: Influence of water activity on growth, metabolic activities and survival of yeasts and molds. *J. Food Prot*., 46, 135-141 (1983)
5. Cysewski, R.G., Wilke, R.C. 1976. Utilization of cellulosic materials through enzymatic hydrolysis. *Biotechnol. Bioeng*. 18: 1297.
6. Haukeli, D.A., Lie, S. 1971. Controlled supply of trace amounts of oxygen in laboratory scale fermentation. *Biotechnol. Bioeng*. 13: 619.
7. Hodge, M.H., Hildebrandt, M.F. 1954. Alcoholic fermentation of molasses. In: Underkofler A.L, Hickey J.R (eds) Industrial fermentation. Chemical publishing Co. NewYork.
8. Hahn-Haegerdal, B., Larrson, M.., and Mattiasson, B. 1982: Shift in metabolism towards ethanol production in *Saccharomyces cerevisiae* using alterations of the physical-chemical microenvironment.12: 199-202.
9. Kordowska-Waiter, M., Zdzissla, T. 2001. Ethanol production on the media containing glucose and xylose by coculture of *Pichia stipitis ccy* 39501 and respiratory deficient mutant of *Saccharomyces cerevisiae v*.Elec. J. Pol. Agr. Univ. 4: 15.
10. Levenspiel, O. 1980. The Monod equation: a revisit and a generalization and product inhibition situations. *Biotechnol.Bioeng*. 22: 1671.
11. Mathewson, W.S. 1980. The manual for the home and farm production of alcohol fuel. In: farm production of alcohol fuel. J. A. Diaz publication.
12. Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. 31: 426-428.
13. Molisson, B. 1993. The permaculture book of ferment and human nutrition. Tagari Publications. Tyalgum.
14. Nowak, J. 2001. Comparisons of polish industrial distillery yeast with ethanol producing bacteria *Zymomonas mobilis. Elec. J. Pol. Agr. Univ*. 4: 6.
15. Park, K.Y., Sato, H.H. 1982. Fungal invertase as an aid for fermentation of cane molasses into ethanol. Applied and environmental microbiology. *American Society for microbiology*. 44: 988989.
16. Raines-Casselman, M.B. 2005. Yeast propagation and maintainence principles and practices. In: The maltose falcons, California.
17. Strand, G. 1998. Science of fermentation. Gert strand AB, Malmo, Sweden.
18. Takeshige, K., Ouchi, K. 1995. Factors affecting the ethanol productivity of yeast in molasses. *Journal of fermentation and Bioengineering*.79: 449-452.
19. Wyman, C.E. and Hinman, N.D. 1990. Fundamental of production from renewable feedstocks and use as transportation fuel. *Appl. Biochem. Biotechnol*. 24/25: 735-75.