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Optimization of parameters for SmF biotransformation of molasses pollutant to alcohol

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Abstract- Parameter optimization, namely molasses contaminant concentration, pH, temperature, and incubation time, was assessed for SmF *in vivo* changes of molasses contaminants to alcohol by *Saccharomyces cerevisiae*-1255. By maintaining the PH of the alcoholic fermentation at 5.1, it has been found that the alcoholic fermentation reaches its highest activity when the 25% molasses contaminant solution is fermented at a temperature of 31°C for 48 hours.

Key words: Alcohol fermentation, *Saccharomyces cerevisiae*-1255, pollutant molasses

INTRODUCTION

Optimization of various parameters[1-6] is performed using either classical or statistical methods. Fermentation medium optimization is generally done in two levels, first screening for the effects of ingredients/nutrients on product formation, then selecting some of the best ingredients/ nutrients and then optimizing them. Further refined.[7] Despite the recent resurgence of interest in fermentation around the world due to the selection and optimization of feed ingredients, with the exception of recent work at CFTRI, Mysore. Depending on the biochemical composition, concentration, incubation time and temperature of each medium, the chemicals have different effects on microorganisms and microbial processes. An important factor for the biosynthesis of industrial alcohol by yeast *S. cerevisiae* the selection of *cerevisiae* strain, selection of bed substrate and its dilution ratio, hydrogen ion concentration of culture medium and production medium, temperature and incubation time, etc. Alcohol production by microbial strains of *Saccharomyces cerevisiae*-1255.

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MATERIALS & METHODS

Medium

The composition of the production medium for SmF conversion of molasses contaminated with *Saccharomyces cerevisiae*-1255 to alcohol is prepared as follows.

Molasses: 25%; Malt extract: 0.275%; Yeast extract: 0.275%; Peptone: 0.450%; $(NH_4)_2HPO_4$: 0.275%; pH: 5.1

Culture medium

To maintain yeast, *Saccharomyces cerevisiae* can maintain metabolic activity. It is cultivated intermittently in Maltagar medium. Fresh medium was prepared every fortnight as follows:

Sucrose: 5%, malt and yeast extract: 0.20 g
Peptone: 0.30g, Agar-Agar: 0.15g
Distilled water: 100 ml, pH: 5.1

Sterilization:

Growth and production media were sterilized in an autoclave maintained at 15 lbs of vapor pressure for 30 minutes. Strain: Saccharomyces cerevisiae-1255 was used in this study. This strain was sourced from NCL in Pune, India. Research method:

The formed alcohol and unfermented molasses contaminants were evaluated by colorimetry.[8-9] Inoculation age: 50 hours. Inoculation volume: 0.5 ml of yeast suspension of Saccharomyces cerevisiae 1255. Molasses concentration: 3%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%. Temperature (in°C): 12, 16, 20, 25, 31, 35, 38, 43, 45 and 50°C. Incubation time: 6, 12, 18, 21, 24, 48, 72, 90, 100 and 105 hours. pH: 1.0, 1.1, 1.2, 2.0, 2.5, 4.0, 4.5, 5.1, 5.2 and 5.5

RESULTS & DISCUSSION

The results obtained from the optimization of various parameters show that the optimum value of SmF conversion of molasses contaminants to alcohol is when the molasses contaminants are fermented at 31°C for 48 hours at 25% (w / v). It shows that it was found and was maintained at 5.1 by the pH of the fermentation medium in the presence of the yeast *Saccharomyces cerevisiae*-1255. The results of alcoholic fermentation by *Saccharomyces cerevisiae* - 1255 in this study generally support the view of conversion of contaminated molasses in alcohol at SmF transformation of molasses pollutant to alcohol 1255 by *S. cerevisiae* becomes easier as the size, molecular weight, and structural complexity of sugar molecules decrease. Since both monosaccharides are slightly associated with phosphorus, it is clear that the simplest sugars, glucose and fructose, are both easy to ferment. The fermentability of galactose is very similar to that of glucose and fructose, but arabinose, rhamnose and sorbose have been found to be the least fermentable. The disaccharide sucrose has been shown to be useful in the production of alcohol, but lactose has been found to be unstable. However, maltose is fermented to some extent based on the total amount of sugar ingested. In the polysaccharide group, starch was the least fermentable and proved unsuitable for alcoholic fermentation. However, raffinose, inulin, and dextrin did not produce alcohol. Polyalcohol mannitol was also unable to produce alcohol by fermentation. The ineligibility of mannitol in fermentation has already been established during the biological conversion to citric acid.[10] Based on the above observations, it can be concluded that glucose in monosaccharides and sucrose in disaccharides are the most suitable and useful for alcoholic fermentation.

In the case of molasses, it was interesting that 7.10 ml of alcohol was produced from the 25% molasses solution. It was used as a starting material for industrial alcoholic fermentation in the course of this study because it is the economical, cheapest and most abundant source of sugar substrate. Various strains of *Saccharomyces cerevisiae* were used as the enzyme source for alcoholic fermentation[11-16], and *S. cerevisiae*-1255 was found to be the most effective and appropriate in this study, so the author selected *S. cerevisiae*-1255 for SmF conversion of pollutant molasses to alcohol. In this study, different molasses concentrations, ie. In other words, 3% to 45% was used for the SmF conversion of molasses pollutants to alcohol, and the 25% molasses pollutant solution (w/v) was found to be the most suitable for biological conversion to alcohol. rice field. Table records two different pollutant concentrations and alcohol yields for molasses. Lower molasses concentrations have proven to be insignificant, and therefore alcohol production has been observed tobe negligible. On the other hand, when the concentration of molasses is high, enzyme activity of cerevisiae is disturbed. It was observed that the SmF conversion of molasses contaminants to alcohol by *S.cerevisiae* 1255 was delayed.

Hydrogen ion concentrations of the production medium also plays vital role in the SmF transformation of molasses pollutant to alcohol. The results of the influence of hydrogen ion concentrations (pH) are recorded in Table 1. It was observed that production of alcohol at the pH values 1.0, 1.1, 1.2, 2.0 and 2.5 was found to be nil. It was further observed that at pH value of 4.0 a low production of alcohol i. e., 3.41 ml/ 100 ml was recorded. From Table 1, it is clear that PH 5.1 is optimal for the production of alcohols (7.15 ml/100 ml) using 25% molasses contaminants as a starting material. It was interesting to note that alcohol production gradually decreased as the hydrogen ion concentration increased from 5.2 to 5.5. From this, it was concluded that a hydrogen ion concentration of 5.1 (Ph) was the most effective and appropriate for the optimal (maximum) SmF conversion of molasses contaminants to alcohol, so this pH was selected and into molasses alcohol. It was maintained on the generation medium of the SmF conversion.

The conversion of molasses contaminants to alcohol is also strongly affected by temperature. The results recorded in Table 1 show that the fermentation yield of alcohol increases with increasing temperature from 12°C to and 31°C. The yield of fermentable alcohol was found to be minimum at low temperatures.Maximum alcohol yields of 2.53 ml / 100 ml at 12°C and 7.10 ml / 100 ml at 31°C were recorded. The fermentation yield of alcohol gradually decreases as the temperature rises from 35°C to 38°C. However, it turns out that higher temperatures are not important for alcohol production. From this, it was concluded that a temperature of 31°C was the most appropriate and effective for the conversion of contaminated molasses to alcohol by *S.cerevisiae* -1255 up to SmF. Therefore, this temperature was used throughout this study in all experiments. It is mentioned in the paper and is retained.

Table 1- Effect of concentration of molasses pollutant, pH, temperature and incubation period on SmF transformation of molasses pollutant to alcohol

% of Molasses pollutant	pH	Temp. in (^{0}C)	Incubation period in hours	Corresponding yield of alcohol*in ml/ 100 ml				Corresponding amount of molasses pollutant*left unfermented in g/ 100 ml			
3	1.0	12	6	0.99	****	2.53	1.32	****	****	5.10	****
5	1.1	16	12	1.28	****	2.81	1.82	****	****	4.81	****
10	1.2	20	18	2.37	****	4.53	2.90	****	****	3.05	****
15	2.0	25	21	3.71	****	5.40	3.25	0.06	****	2.01	****
20	2.5	31**	24	5.19	****	7.17**	3.92	0.04	****	0.79	3.62
25**	4.0	35	48**	7.10**	3.41	5.59	6.84	0.82	4.16	1.73	0.69
30	4.5	38	72	7.56	5.43	4.61	6.32	****	2.18	****	0.83
35	51**	43	90	7.80	7.15**	****	5.31	****	0.72	****	0.73
40	5.2	45	100	****	6.42	****	****	****	1.10	****	****
45	5.5	50	105	****	5.31	****	****	****	1.08	****	****

* Each value represents mean of three trails
* Optimum values of molasses pollutant solution, pH, temp. and incubation period.
*** Optimum yield of alcohol
**** Insignificant value.

Incubation time plays an important role in the fermentation process as it is directly related to the industry's excellent economics. The results recorded in Table show that the alcohol yield increases with increasing incubation time from 6 hours to 48 hours, followed by a gradual decrease in alcohol yield (72 to 90 hours incubation time). Long incubation times proved to be insignificant, so 100 and 105 hours of incubation were discarded. Investigations into the effects of different incubation times on alcohol yields from 25% molasses contaminants show that this happens at different stages. The first phase is completed in 12 hours. In the meantime, slower molasses consumption results in lower alcohol yields. The next second phase occurs 18 hours and 24 hours of incubation period where molasses pollutant consumption and yields of alcohol follows the first phase with slight improvement in the yield of alcohol. After 24 hours of incubation period that the 3rd important and effective last phase begins and the alcohol yields are maximum in this very phase, i.e., in 48 hours. In this way 48 hours of incubation period gives the maximum yield of alcohol, that is 6.84 ml/ 100 ml. thus, SmF transformation of molasses pollutant to alcohol by *S. cerevisiae* – 1255 was optimized using 25% molasses pollutant, 5.1 PH, 31°C temperature and 48 hours of incubation period along with some other necessary growth ingredients.

REFERENCES

1. **Mongahan R. L., & Koupal L. R. 1989.** Novel microbial products for medicine and Agriculture, *Elseiver New- York.* 25-32.
2. **Young M. D., Kempe L. L. and Bader F. G. 1985.** Effects of phosphate, glucose, and ammonium on cell growth and lincomycin production by *Streptomyces lincolnensis* in chemically defined media. *Biotechnology and Bioengineering.* **27(3):**327-333.
3. **Holt G., Saunders G., Demain A. L., Solomon N. A. (eds). 1986.** Manual of Industrial and Microbiology and Biotechnology, *American Society of Microbiologists, Washington D. C.* 41-48.
4. **Zabriskie D.W., Armiser W. B., Arbano D.A., & Phillips D. H. 1988.** Trader's guide to fermentation, *media, Formulation trader's Protein Mephis Tenn.*
5. **Srinivas M. R.S., Chand N., & Lonsane B. K. 1993.** *Bioprocess Eng.***10:**139.
6. **Greasham R.L., Reed H. J., Rehm G. P. 1986.** *Bioprocessing VCH Publishera A. and Stadler, P. (eds.) Inc., New- York.* 128-139.
7. **Greasham R. L., & Inamine E. 1986.** Manual of Industrial microbiology and Biotechnology. *American Society of Microbiologists, Washigton D.C.* 41-48.
8. **Mccloskey L. P., & Replogle L. L. 1974.** *Am. J. Enol Vitie.* **25:**194.
9. **Dubois M., Gilles K. A., Hamilton J. K., Rebers P. T. and Smith F. 1956.** Colorimetric method for determination of sugars and related substances. *Analytical chemistry*. **28(3):**350-356.
10. **Singh S. P., Kumar B., Pratap B., Pandey A.K., & Srivastava S. K. 1992.** *Proc. VIII Cabohydrate Conf. Nov.Trivandrum.*173-175.
11. **Krumphazal V., Josef D. & Strejeck J. 1964.** *Czech.* **116:**443.
12. **Singh S. P., Kumar B., Pandey R. K., & Bihari A. 1992.** *Biojournal*. **4(1&2):** 245.
13. **Singh S. P., Sinha A. P., Prasad M., Kumar L., & Yadav S. K. 1992.** *Biojournal.* **4(1&2):** 241.
14. **Singh S. P., Pratap B., Ambastha B. K., Pandey R. K., Sinha A. C., & Prasad A. 1994**. *Asian J. Chem.* **6:**753.
15. **Singh S. P., Brahmachari A. K., Singh M.K., & Kamal P. 1997.** *Asian J. Chem.* **9:**157.
16. **Singh S. P., Ambastha B. K., &Rathor N. 1998.** *Asian J. Chem.***10:**375.

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