Optimization of Physical Parameter for Bioethanol Production from Water Hyacinth (Eichhornia Craseeipes) By Ethanol and Temperature Resistant Strain of Saccharomyces Cerevisiae

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Abstract - Water hyacinth is promising plant for the production bioethanol. The water hyacinth was saccharified with dilute alkali to fermentable sugar under high pressure (20 psig). The present study was focussed on whether the reducing sugar content of saccharified water hyacinth hydrolysed can be used as a carbohydrate raw material for the production of alcohol. The influence of various physical parameters such as particle size, pulp densities, initial pH of the medium, time period of fermentation, temperature, volume of medium, age of culture and cell density on ethanol production by Saccharomyces Cerevisiae AB_{810} were studied.

Key words: Water hyacinth, Bioethanol, Physical parameter.

I. INTRODUCTION

In view of increasing importance of ethanol as an alternative resource for chemical and liquid fuel, a great deal of research interest in ethanol fermentation has been generated in recent years (Wayman et al., 1990; Chandel et al., 2010). In India, due to the rising cost of petroleum and the rate of depletion of fluid fossil fuel resources, it is necessary to produce more and more ethanol from agricultural carbohydrate products and biomass to satisfy the fuel combustion demand and other chemical industrial need. Water hyacinth first growing plant is potential source for production a usable grade of ethanol. Water hyacinth is considered as an alternative raw material for the production of fuel ethanol because of its availability in large quantities at low costs (Estigachi et al., 2012).Water hyacinth grows rapidly and produces almost two tons of biomass per acre of land and population doubles every 10 to 15 days(Craft et al.,2003).

Studies in our laboratory indicated that the ability of physical pretreatment on water hyacinth for improving the yield of sugar and processing time in dilute alkali hydrolysis with high pressure (20 psig) is better than acid pretreatment process (Biswas A. B. et al., 2015).We have also developed a high yielding ethanol and temperature resistant strain of Saccharomyces Cerevisiae to increase the rate of production of ethanol from sugar obtained from hydrolysed water hyacinth cellulose(Biswas Biswanath et al.,2015).

The present study was concentrated on the fermentation of these reducing sugar obtained from alkali hydrolysis of water hyacinth under high pressure to alcohol and optimization of various physical parameters such as particle size of powdered water hyacinth, substrate concentration, initial pH of the medium, temperature, period of incubation, volume of the medium, age of the culture and cell density of Yeast.

II. MATERIAL AND METHODS

Raw materials: Fresh water hyacinth plant with long steam was collected from natural pond (Sonarpur, 24 south parganas, West Bengal) (Biswas Biswanath et al., 2015). The water hyacinth was thoroughly washed in several times with tap water to remove adhering dirt. Then was chopped in small pieces and finally it dried in hot air oven at 80 ^{0}C with circulation of air for 8 hours. Fermentable sugar present in the fermentation medium was obtained after physical pretreatment of dried water hyacinth with dilute alkali (5 % NaOH) of 20 psi pressure for 30 min. The best pretreatment method concluded in this study was combination of drying, grinding, steaming with high pressure (20 psig) with yield 97.50% sugar in alkali which is higher than acid pretreatment process 80.60 %(Biswas Biswanath et al., 2015).

Microorganism: The yeast Saccharomyces cerevisiae AB used in this study was isolated from instant yeast supplied by Khotari Fermentation and Biochem Limited Company, New Delhi (India).This strain was used for the development of high alcohol resistant (15%) and temperature resistant(35 ^{0}C) strain. This alcohol and resistant strain of Saccharomyces cerevisiae AB_{810} was used with present investigation of different physical parameters.

Medium and cultural conditions: The alcohol and temperature resistant strain of Saccharomyces cerevisiae

AB_{810} was maintain in YPD agar medium containing of yeast extract 1%, peptone 2%, dextrose 2% and agar 4%, pH was adjusted to 4.5 slant at 4 ^{0}C.The fermentation medium used for alcohol production contained glucose 5%(obtained from hydrolysed water hyacinth), KH_2PO_4 0.1%, $NaNO_3$ 0.2%, $MgSO_4$.7 H_2O 0.05%,Yeast extract 1% and pH 4.5.The yeast cells were harvested by washing the slant with sterilized distil water and filtering the resulting cell suspension through several layers of absorption cotton. The cell density was adjusted to 2.6×10^7cell/ml of the suspension .The cell suspension was used for the inoculation of fermentation medium. Surface culture fermentation was carried out using 500 conical flasks each containing 200 ml of medium. The flasks were then incubated at 28 ^{0}C for 48 hours.

Determination of ethanol concentration: After alcohol fermentation the ethanol produce was determining by gas chromatography (GC) (Pye Unicam series ion) with flame ionization detector (FID) on a column of porapak-O using N_2 as carrier gas. The column temperature and detector temperature was 190 ^{0}C and 230 ^{0}C respectively. In each case 5 µL sample was injected. The quantitative calculation of ethanol concentration was made by measuring the peak areas of sample in calibration relative to the interval standard n-Propanol used as internal standard (Mobini-Dehkordi et al., 2007).

Fermentable and non fermentable glucose was measured by AOAC method (AOAC, 1950).

III. RESULT AND DISCATION

Effect of particle size: In the present study, the maximum alcohol production occurred with the particle size of dried water hyacinth 30 to 40 mesh, with increase or decrease in size of the substrate, the production of alcohol decrease (Table:1).Smaller particle size of dried water hyacinth was more susceptible to microbial attack than the larger size because with decreasing particle size, surface area of the substrate particles increased and hence better contact occurred(Ghosh Runa et al.,1998).Fine mesh size of the dried water hyacinth particles will result in greater surface area which aid maximum alcohol production.

Table 1: Effect of particle size on Bioethanol production from water hyacinth by Saccharomyces cerevisiae AB_{810}.

S. No.	Particle size (mesh)	Ethanol production % (w/v)
1	-10 to -20	5.5
2	-20 to 40	6.7
3	-40 to- 60	6.0
4	-60 to -80	5.5
5	-80 to- 100	5.5

Effect of pulp density: The quantity of alcohol production reached a maximum with the pulp density (dried water hyacinth)of 5%.It is seen that higher or lower the quantity of water hyacinth beyond 5%.there was lowered the production of alcohol.(Table:2)

Table 2: Effect of pulp densities on Bioethanol production from water hyacinth by Saccharomyces cerevisiae AB_{810}.

S. No.	Pulp density %	Ethanol production% (w/v)
1	3.0	5.0
2	4.0	6.7
3	5.0	7.0
4	6.0	5.8
5	7.0	5.0

Effect of initial pH: Initial the pH of the production medium was adjusted using 0.1(N) NaOH and 0.1 (N) HCl. The effect of pH on alcohol production was examined among different levels (3.5 to 6.0) of initial pH of the media. Production of alcohol was maximum (7.4%) at pH 5.0 by Saccharomyces cerevisiae AB_{810} (Fig. 1) with farther increase in pH production decreased.

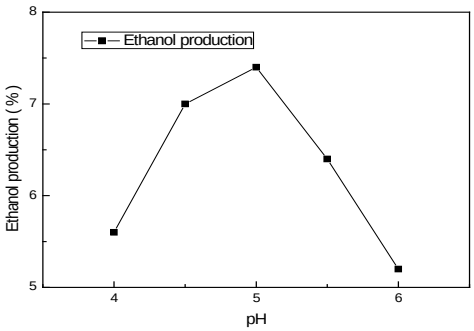

Fig. 1 Effect of initial pH of the medium on Bioethanol production

Effect of period of incubation: To determine the optimum period of incubation for alcohol production the culture media were incubated for different period from 24 to 72 hour and it was observed that at 48 hour incubation the production of alcohol was maximum (Fig. 2).

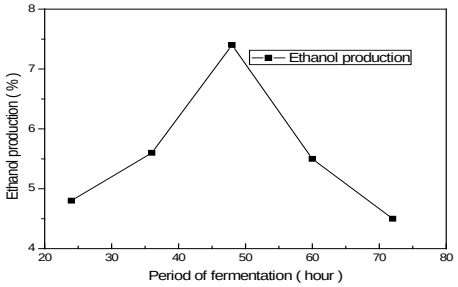

Fig. 2 Effect of time period of fermentation on Bioethanol production

Effect of temperature: The effect of wide range of operational temperature (25^0 to 30^0C) on alcohol production from water hyacinth was carried out in flasks. At 28 ^{0}C maximum yield of alcohol (7.4 %) was obtained by the experimental strain Saccharomyces cerevisiae AB_{810}.Temperature also plays an important role in growth rate of microorganism.

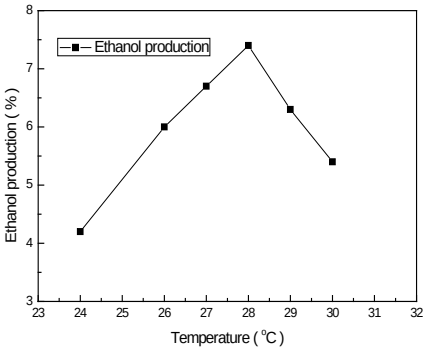

Fig. 3 Effect of temperature on Bioethanol production

The shift in temperature can after the utilization rate of one component as compared to other, thus unbalance the media with respect to growth (Fig. 3).

Effect of different volume of medium: To optimize the volume of the medium, five different volumes were examined. After 48 hour of incubation, it was observed that alcohol productions highest for 200ml medium contained in a 500 ml Erlenmeyer flask. Too high media volume may dilute the alcohol content whereas too low a volume may give insufficient nutrient for the growth of the microorganism production of desired metabolite declines sharply (Table: 3).

Table 3: Effect of different volume of medium on Bioethanol production from water hyacinth by Saccharomyces cerevisiae AB_{810}.

S. No.	Volume of medium (ml)	Ethanol production % (w/v)
1	100	5.2
2	150	6.7
3	200	7.4
4	250	6.5
5	300	5.6

Effect of the age of the culture: To study the effect of age of inoculums five different ages (24 to 72 hours) of inoculums were studied to yield maximum amount of alcohol. Maximum efficiency to produce alcohol by the yeast was observed at 48 hour age. It is probably at the late lag phase and early lag phase of growth, the rate of alcohol production is highest by yeast.24 hour might too early to reach at the late lag phase and more than 60 hours as too late and the growth of the yeast may go to the late lag phase or stationary phase(Table : 4).

Table 4: Effect of age of culture on Bioethanol production from water hyacinth by Saccharomyces cerevisiae AB_{810}.

S. No.	Age of culture (hour)	Ethanol production % (w/v)
1	24.0	3.5
2	36.0	5.4
3	48.0	7.4
4	60.0	6.4
5	72.0	6.0

Effect of cell density of inoculum: Five different cell densities of inoculum were studied to optimize the cell density needed for the maximum alcohol production by Saccharomyces Cerevisiae AB_{810}.The maximum alcohol production was observed at 3.0×10^7cell/ml of inoculum size .Above and below 3.0 × 107cell/ml alcohol productions was decreased gradually. Too low a cell density may give insufficient biomass and too high density may produce too much biomass and deplete the substrate of nutrient necessary for alcohol fermentation (Table: 5).

Table 5: Effect of cell density of inoculums on Bioethanol production from water hyacinth by Saccharomyces cerevisiae AB_{810}.

S. No.	Cell density of inoculums(10^7cell/ml)	Ethanol production % (w/v)
1	2.0	6.0
2	2.6	7.4
3	3.0	8.4
4	3.5	6.8
5	4.0	5.8

IV.CONCLUSION

Therefore, from our present experimental study, it was concluded that using minimum salt medium, by optimizing different physical parameters, production of alcohol was increased from 6.7 to 8.4%.by the alcohol and temperature resistant strain of Saccharomyces cerevisiae AB_{810}.

V. FUTURE SCOPES

Further studies are in progress to assess the nutrient requirements of the yeast and to make the whole process industrially feasible.

VI.ACKNOWLEDGEMENTS

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