J. Indian Chem. Soc.,
Vol. 85, June 2008, pp. 665-667

NOTE

Potential utilisation of aquatic weeds for the production of alcohol

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Manuscript received 28 August 2007, revised 7 February 2008, accepted 27 March 2008

Abstract : The rhizomes of *Typha angustata, Cyprus exaltatus,* aquatics weeds which are rich in carbohydrates were processed to generate the fermentable sugars. The fermentable starch hydrolysates were used as fermentation substrates for the yeast *Saccaramyces cereviceae*. The optimum conditions for the survival, growth and fermentation of the yeast *Saccaramyces cereviceae* with the selected substrates was evaluated.

Alcohol was produced on a pilot scale by fermentation using these two substrates.

Keywords : Alcohol production, starch, rhizomes, *Typha angustata, Cyprus exaltatus, Saccaromyces cereviceae.*

Introduction

The current interest on the economic conversion of renewable resources into alcohol has paved way to the evaluation of a number of starchy crops for alcohol production[1-4]. Over the past few years sugarcane crops were the sources of substrate for alcohol biosynthesis[5]. But a possible way to make fermentation more cost effective is to use lignocellulosic feed stocks which are available in wood and agricultural wastes as they are available in large quantities. One such attempt was made in the present investigation using yeast[6-8]. Two water plants namely *Typha angustata* and *Cyprus exaltatus* were chosen as substrates for the fermentation of alcohol. Rhizomes of these two plants were found to be rich in starch and sugar. These crops are photosynthetically efficient[6] which grow through out the hotter parts of India. Hence it was decided to use these plants as a source for alcohol production by fermentation using the yeast *Saccaromyces cereviceae*. The species of *Typha angustata* and *Cyprus exaltatus* used for the study were obtained from a nearby place to Alwarkuruchi where there was surplus of water stagnation.

Results and discussion

Results of the hydrolytic and fermentation studies :

Enzymatic hydrolysis of the substrates at pH 6.0 was found to produce good quantities of reducing sugar. The optimum temperature for hydrolysis was found to be 40 °C. The amounts of reducing sugars produced under the above conditions with *Cyprus exaltatus* was 8.9% and with *Typha angustata* was 7.2% (the hydrolytic action of amylase was maximum after 12 h of treatment).

The growth condition of the yeast strain *Saccaromyces cereviceae* in the fermentation media prepared out of *Typha angustata* and *Cyprus exaltatus* were optimised with respect to temperature and pH. The optimum pH is 6.0 with both the substrates. The optimum temperature for growth was 40 °C with both the substrates[9-11]. The pH of the fermentation media were adjusted to 6.0 that was favorable for the strain used in the study. Fig. 1 shows the response of the microbe at various pH values and Fig. 2 reveals the temperature response of the strain with the selected substrates.

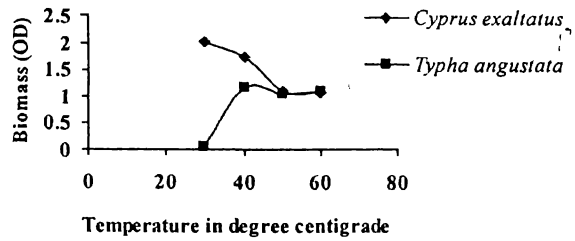


Fig. 1. Response of the microbe at various temperatures.

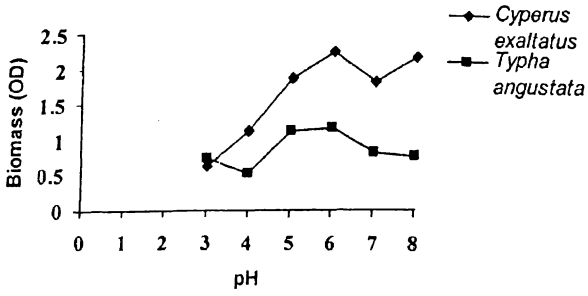


Fig. 2. Response of the microbe at various pH values.

Table 1. Results of hydrolytic and fermentation studies with *Typha angustata* and *Cyprus exaltatus*

Parameter	Process	*Typha angustata*	*Cyprus exaltatus*
pH		6	6
Temperature		40	30
Quantity of sugar produced	Hydrolysis	7.2%	8.9%
pH		6	6
Temperature		40	30
Quantity of alcohol produced	Fermentation	14.0	12.9%

Table 1 summarises the results of the hydrolysis and thereafter fermentation studies.

We are therefore successful in using aquatic weeds as a choice of substrate for alcohol production. The chief attraction of the chosen substrates is their utilisation without having to dewater them. *Saccaromyces cereviceae* could be used as the choice of microbe for the fermentation of the selected aquatic weeds *Typha angustata* and *Cyprus exaltatus*. This reinstates the relevance of yeast in waste recycling and generating value added products for the fermentation process. The substrates used were only partially analysed for its chemical nature. With details of the complete analysis it could be possible to produce a more efficient substrate for ethanol production.

Materials and methods :

The morphological view of *Typha angustata* and *Cyprus exaltatus* used in the present study are shown in Figs. 3 and 4.

The rhizomes of these crops were washed nicely crushed and a slurry was prepared using them with distilled water at 1 : 5 ratio. The starch present in the rhizome was enzymatically hydrolysed with 0.6% α-amylase. For efficient hydrolysis of starch the slurry with enzyme was incubated at 95 °C for 30 min and filtered through Whatman No. 1 filter paper. pH and temperature

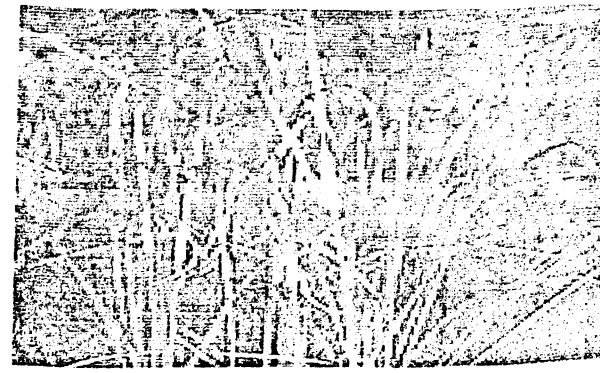


Fig. 3. *Typha angustata*.



Fig. 4. *Cyprus exaltatus*.

of the hydrolytic media during hydrolysis were modified to find out the appropriate pH and temperature.

Strain specification :

Saccaromyces cereviceae MTCC used for the present work was obtained from the microbial culture collection centre, Chandigarh, India. The organism was maintained in yeast peptone dextrose medium (YPD) and sub cultured regularly. Before the initiation of fermentation with the above prepared media, the conditions best suited for the microbial growth was optimized with both the substrates.

Inoculum preparation :

The yeast inoculum was prepared by suspending a loopful of yeast from a fresh slant culture to 50 mL of sterile yeast peptone dextrose broth. This was incubated at room temperature overnight. From the overnight culture 1.0 mL seed was taken and 9.0 mL sterile YPD medium was added and centrifuged at 1000 rpm for 10 min. The supernatant was discarded and 1.0 mL of the yeast pellet was added to the fermentation media whose pH was earlier adjusted to 6.0.

Batch fermentation :

The hydrolysed starch media were diluted and inoculated with yeast. Fermentation was allowed to proceed at room temperature for 12–24 h. Samples for analysis were withdrawn at 6 h intervals.

Analysis of fermentation media :

Ethanol produced by fermentation was first separated out from the media by distillation and the obtained distillate was analysed for its alcohol content. Known aliquots were treated with chromic acid, warmed to 80 °C for 15 min and cooled down to room temperature. The colour developed was measured at 530 nm[7] using a spectrophotometer. A calibration chart was prepared using known concentrations of alcohol. The amount of ethanol produced on fermentation was deduced from the calibration graph. Reducing sugar present in the fermentation media were analysed by DNSA method.

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