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# Microbial production of ethanal from water hyacinth

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Vast quantity of the aquatic weed water hyacinth which is available throughout the year at no cost, has prompted studies on its use as renewable carbon source for fuel ethanol production. In order to use this lignocellulosic biomass, its carbohydrate polymers (cellulose and hemicellulose) was first hydrolyzed (by enzymatic hydrolysis) with crude cellulase enzyme produced on site on the pretreated, ground water hyacinth biomass in liquid culture. The hydrolysate was then fermented successively with glucose and xylose fermenting yeasts *(Saccharomyces cerevisiae* and *Pachysolen tannophilus* respectively) to obtain ethanol.

**Key words :** Water hyacinth, *Trichoderma reesei,* fermentation, *Saccharomyces cerevisiae, Pachysolen tannophilus,* ethanol

## INTRODUCTION

Water hyacinth is a hazardous aquatic weed, growing extensively in several parts of tropic and sub-tropics. Due to high rate of propagation it causes huge economic loss. On the other hand, it has unique ability to adsorb heavy metal pollutants (Cd, Cr, As, Eu) from waste water onto their roots. Hence, the plant is currently being used throughout the world in waste water treatment. Vast quantity of this weed which is available throughout the year at no cost, has prompted studies on its use as renewable carbon source in various bioconvertion processes, as production of fuel or myco-protein rich food and feed. In the present investigation this plant biomass (without root) has been used as a carbon source for fuel ethanol production. In order to use this lignocellulosic biomass, its carbohydrate polymers (cellulose and hemicellulose) need to be first hydrolyzed (preferably by enzymatic hydrolysis) into their respective monomers (glucose and xylose respectively). This hydrolysate in turn can be used as carbon source for yeast fermentation to produce ethanol. The present paper deals with the optimization of some basic factors affecting fermentation of water hyacinth hydrolysate.

## MATERIALS AND METHODS

### *Substrate*

Water hyacinth [*Eichhornia crassipes.* (Mart.) Solm., family Pontederiaceae] was collected from local stagnant ponds, and chopped into small pieces. The plant (without root) pieces were pretreated following Mukhopadhyay and Nandi (2001) to use as pretreated water hyacinth (PWH).

### *Microorganisms*

The fungus *Trichoderma reesei* ATCC 26921 (courtesy U.S. Dept. of Agriculture. Peoria, Ilinois) was used as efficient cellulase producer. The glucose fermenting yeast *Saccharomyces cerevisiae* MTCC 171 and the xylose fermenting yeast *Pachysolen tannophilus* MTCC 1077 (courtesy, IMTECH, Chandigarh) were used to ferment water hyacinth (lignocellulose) hydrolysate.

The enzyme production by *T. reesei* was done by growing fungus for 8 days at 31±1°C in a very simple liquid medium containing PWH as the sole carbon source as optimized by Mukhopadhyay and Nandi (1999). The culture filtrate was used as enzyme source to hydrolyse 4%(w/v) PWH at 45°C for 72 hrs.

### *Fermentation*

Nutrient-rich yeast fermentation medium (YFM) was added to hydrolysate and total fermentation medium was inoculated with suspensions of *S. cerevisiae* (2% v/v at a concentration of approximately $10^8$ cells/ml) and *P. tannophilus* (3% v/v at a concentration of approximately $6 \times 10^7$ cells/ml) either simultaneously or at 24 – 42 hrs. intercals and incubated at different temperatures for different periods to optimize favorable conditions. Samples were withdrawn from fermentation broth at 24 hrs intervals to collect ethanol solution by fractional distillation at 78–79°C. Concentration of ethanol in the distillate was then estimated spectrophotometrically following Caputi *et al.* (1968).

Some basic factors affecting fermentation process were studied to determine most favorable conditions.

### *Effect of fermentation period*

The total fermentation medium was inoculated with suspension of *Saccharomyces cerevisiae* and incubated at 30°C for different period between 24 to 120 hrs to obtain the optimum period.

### *Effect of inoculation of P. tannophilus*

For fermentation of xylose sugar (obtained from hemicellulose degradation) in the hydrolysed broth, the xylose-fermenting yeast *P. tannophilus* was also inoculated to the fermentation liquid either simultaneously or at 24–48 hrs intervals of *S. cerevisiae* inoculation and incubated at their ambient temperatures.

### *Effect of agitation*

During incubation, fermentation broth (within flask) was agitated at 150 rpm for varying periods after inoculation of each species.

## RESULTS AND DISCUSSION

### *Enzyme production and enzymatic hydrolysis*

Enzyme produced by *T. reesei* ATCC 26921 on PWH in submerged shake culture showed considerable cellulase (0.24 IU/ml filter paper cellulase), avicelase (2.0 IU/ml) as well as xylanase (2.2 IU/ml) activities. This crude cnzyme was used to hydrolyze pretreated water hyacinth (at 45°C and for 72 hrs) in which cellulose became more exposed and thereby more susceptible to enzymatic action due to selective removal of greater proportion of hemicellulose and part of lignin by chemical pretreatment.

The product array of the hydrolysate analysed through GLC revealed the presence of glucose (12.5 g/l) and xylose (3.2 g/l) as the two most predominant sugars along with small amount of arabinose, mannose and galactose (released from hemicellulose hydrolysis).

**Table 1 :** Effect of fermentation period

| Fermentation period (hrs) | Concentration of ethanol (g/l) |
|---|---|
| 24 | 3 |
| 48 | 3.8 |
| 72 | 4 |
| 96 | 4.5 |
| 120 | 4.1 |

**Table 2 :** Effect of conditions of inoculation of yeast species

| Conditions of inoculation of yeast species | Concentration of ethanol (g/l) |
|---|---|
| Simultaneous inoculation of *S. cerevisiae* & *P. tannophilus* | 4.5 |
| Successive inoculation of *S.cerevisiae* & *P. tannophilus* at 24 hrs interval | 4.9 |
| Successive inoculation of *S. cerevisiae* & *P. tannophilus* at 48 hrs interval | 5.1 |
| Successive inoculation of *S. cerevisiae* & *P. tannophilus*, at 72 hrs interval | 5 |

**Table 3 :** Effect of agitation of fermentation broth

| Conditions of agitation of fermentation broth | Concentration of ethanol (g/l) |
|---|---|
| No agitation | 5.1 |
| Agitation for 96 hrs | 5.6 |
| Agitation for first 24 hrs after inoculation of each yeast species | 5.8 |

***Fermentation***

The common yeast *S. cerevisiae* was employed to ferment glucose the main constituent of above cellulose hydrolysate. *P. tannophilus* was used as an yeast capable of fermenting both glucose and xylose.

After studying some basic factors such as affect of different fermentation period, inoculation of *S. cerevisiae* and *P. tannophilus* at different intervals, agitation of fermentation broth for different periods, a maximum ethanol concentration of 5.8 g/l was obtained with the following conditions : (i) after a maximum period of fermentation for 96 hrs with *S. cerevisiae;* (ii) successive inoculation of *S. cerevisiae* (at 30° C) and *P. tannophilus* (at 25° C) suspensions at 48 hrs interval; and (iii) agitation of fermentation broth for first 24 hrs after inoculation of each yeast species.

Thus the hazardous aquatic weed water hyacinth, which is known to play a role in waste water treatment, could also be effectively used as cheap, renewable feed stock in biological production of fuel ethanol.

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