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DIRECT BIOCONVERSION OF RICE STRAW AND BAGASSE TO ETHANOL BY *FUSARIUM OXYSPORUM*

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ABSTRACT

Effect of substrate size for the direct bioconversion of rice straw and bagasse to ethanol by Fusarium oxysporum was studied in a batch reactor at constant temperature and pH. Maximum ethanol concentration was obtained for 100 mesh size screen in eight days when the substrates were pre-treated with alkali. It was found that the ethanol concentration was higher for bagasse when compared to rice straw. Maximum ethanol concentration was obtained with a substrate concentration of 2% in 100 mesh size scree for bagasse and ricestraw were found to be 10.59 g/L and 8.6 g/L, respectively. The kinetic data were fitted with logistic growth model and Leudeking-piret model and it was found that the former model closely represents the fermentation process whereas Leudeking-piret model represents the fermentation process only at the initial stages.

Key words: Bioethanol, Lignocellulose, *Fusarium oxysporum*, Product formation kinetics.

INTRODUCTION

Energy consumption has been increasing steadily with the population growth and industrial development. Conventional energy sources have difficulty in meeting the increasing energy demand. Therefore, there is great interest in exploring the alternative energy sources to maintain the sustainable growth of society[1]. The potential source for low-cost ethanol production are to utilize lignocellulosic materials such as crop residues, grasses, saw dust, wood chips and solid animal waste. Lignocellulosic materials constitute a major part of available biomass in nature. Their main components are cellulose (25-53%), hemi cellulose (20-35%), lignin (10-25%) and other extractives. Bagasse and Rice straw consists of 55.1% cellulose and 46% cellulose respectively. In comparison with other agricultural residues, bagasse can be considered as rich solar energy reservoir due to its high yields and annual regeneration capacity[2,3]. The utilization of lignocellulosic biomass for fuel ethanol is still under development. Direct bioconversion of cellulose to ethanol is a process in which the same microorganism carries out both hydrolysis and fermentation of cellulose to ethanol in one operation[4]. A few microbial species have been reported to acquire the ability of fermenting cellulose directly to ethanol. Among fungi, strains of *Fusarium* species have been reported to ferment cellulose to ethanol in a one-step process, but no

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details on yields have been published. *Fusarium oxysporum*, on the other hand, fermented pentoses to ethanol with high yield[5].

The present paper reports the findings of the experiments for the direct bioconversion of bagasse and rice straw to ethanol by *Fusarium oxysporum*.

EXPERIMENTAL

Materials and Methods

Fusarium oxysporum was obtained from the institute of microbial technology, Chandigarh. The stock culture was maintained on potato-sucrose-agar.

The production medium had the following composition per liter of distilled water: KH_2PO_4, 2g; $MgSO_4$, 0.3g; $CaCl_2$, 0.3g; peptone, 5g; yeast extract, 3g; malt extract, 3g; $FeSO_4.H_2O$, 0.05g; $ZnSO_4.4H_2O$, 0.014g; $MnSO_4.4H_2O$, 0.016g; $CoCl_2$, 2g and substrate. The pH of the medium was adjusted to 6.0.

Substrates used in these studies were rice straw and bagasse. Pre-treatment of bagasse and rice straw has often been found useful to improve its digestibility and easy access for microbial attack. Also the pre-treatment gives the enlargement of the inner surface area of substrate particles accomplished by partial solubilization and degradation of hemi cellulose and lignin[6]. Among several pre-treatment methods, chemical pre-treatment with alkali such as sodium hydroxide solution have been found effective and economical. Substrates were first dried at 100^{o}C for one day, crushed, milled for 2 hrs and sieved before pre-treatment. The substrates were sieved through various mesh sizes namely 60, 100 and 240 respectively. The substrates were treated with 5% sodium hydroxide at room temperature for 8 h. The mixture was filtered and the residue was washed with water. The moisture content of raw and treated substrates was found to be 7 ± 0.5%. The improved digestibility of cellulose content of bagasse and rice straw after pretreatment with sodium hydroxide were found to be 73.4% and 50% respectively.

Batch fermentations were run in ten sterile 250 mL conical flasks with cotton wool plugs containing 100 mL of production medium. 2 % (v/v) of inoculum medium was transferred to each 100ml production medium in sterile conditions. The pH was initially adjusted to 6.0 and the inoculated flasks were maintained at 30^{o}C in a rotary shaker at 150 rpm. Samples were removed and analyzed periodically for the determination of ethanol concentration and dry cell mass.

Effect of substrate size on the production of ethanol was studied by conducting the experiment with different mesh size (60, 100 and 240). The experiments were carried out for the fermentation period of 9 days with regular analysis for a substrate concentration of 2% (w/v) at 30^{o}C and inoculum size of 2% (v/v).

Effect of substrate concentration on ethanol production was studied at constant temperature for different initial substrate concentrations (1%, 2%, 4% and 6%) for the fermentation period of 8 days at pH 6.0 and inoculum size 2% (w/v).

Cellulose was determined by the Anthrone reagent method[7]. Ethanol was estimated using gas chromatograph with FID detector. The column used was poropak. Cell mass was determined by centrifuging the samples. The settled biomass was collected after centrifugation and dried. The weight of the dry cell mass was determined.

RESULTS AND DISCUSSION

Certain growth parameters such as pH, temperature and substrate concentration significantly affected ethanol production. The effect of substrate size on ethanol production is shown in Figs. 1 and 2. Fig 1 shows that highest ethanol concentration was obtained for bagasse when compared to rice straw.

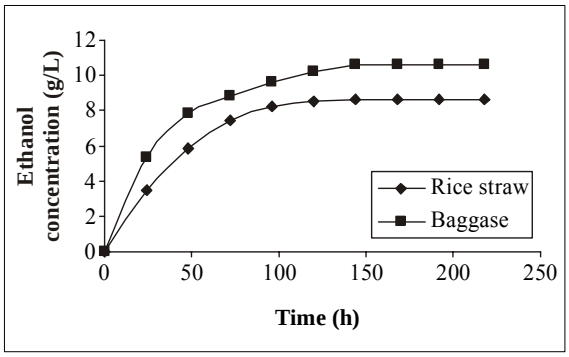

Fig. 1: Ethanol production by *Fusarium oxysporum*

Maximum ethanol concentration of 10.59 g/L was obtained in 8 days for Bagasse and 8.6 g/L of ethanol concentration was obtained for Rice straw when the substrates were pre-treated with alkali.

From Fig. 2, it was concluded that the maximum production of ethanol was obtained when the mesh size was 100 which has the average diameter of 0.1965 mm with a substrate concentration of 2% (w/v) in 8 days.

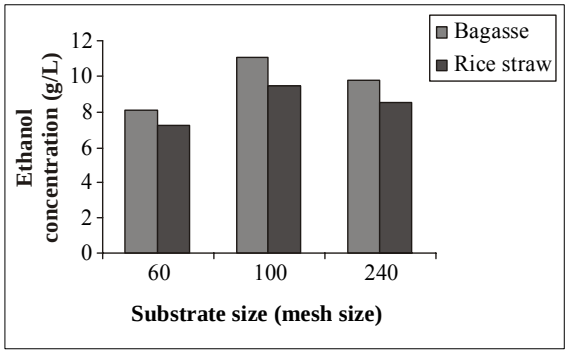

Fig. 2: Effect of substrate size on ethanol production

High ethanol yield and easy access of microorganisms to cellulose was achieved at low initial substrate sizes.

The effect of substrate concentration on the production of ethanol appears in Fig. 3.

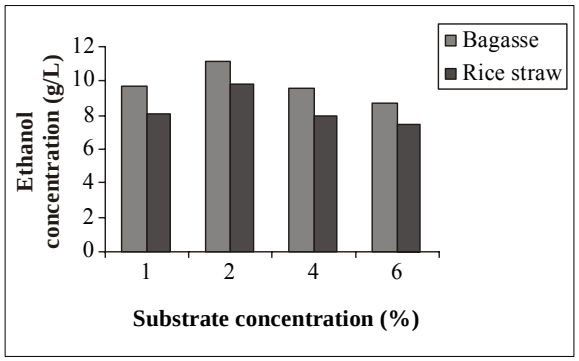

Fig. 3: Effect of substrate concentration on the production of ethanol

Increasing the substrate concentration from 1% (w/v) to 6% (w/v) lowers the ethanol concentration although a higher yield of ethanol was obtained for 2% (w/v). The decreased conversion may be due to insufficient amount of Fusarium oxysporum mycelial biomass used for fermentation and also probably due to the inhibition of fermentation by the compounds resulting from sugar and lignin degradation in rice straw and bagasse.

Kinetic models

The simplest types of product formation kinetics arise when there is a simple stoichiometric connection between product formation and substrate utilization of cell growth[8].

The logistic equation for growth kinetics is given by -

$$x = \frac{x_o e^{kt}}{1 - \beta x_o \left(1 - e^{kt}\right)} \qquad \ldots(1)$$

where $x_s = 1/\beta$

The Leudeking-piret model for the product formation kinetic is -

$$P(t) - P_o - \beta\left(\frac{x_s}{k}\right)\left[1 - \frac{x_o}{x_s}\left(1 - e^{Kt}\right)\right] = \alpha\left[x(t) - x_o\right] \qquad \ldots(2)$$

where P(t) is the product concentration at any time t (g/L), P_o, the initial product concentration (g/L), x_s, the biomass concentration in stationary phase (g/L), x_o, the initial biomass

concentration (g/L), x(t), the biomass concentration at any time (g/L) and β, α & k, the constants.

The kinetic data obtained for rice straw were used to evaluate the model parameters in the Logistic and Leudeking-piret kinetic model. The values of k, α, β obtained were found to be 0.08114, 0.65 and 0.0003 respectively. The simulated results were compared with the experimental results and are given in Figs. 4 and 5.

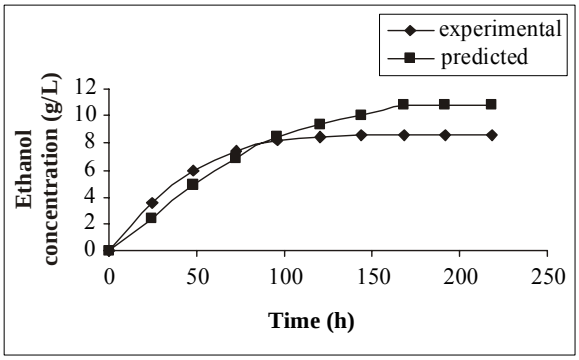

Fig. 4: Comparison on between experimental results and product form at ion kinetic model

The logistic model is a reasonable representation of the fermentation process and the leudeking-piret model represents the fermentation process only at the initial stages.

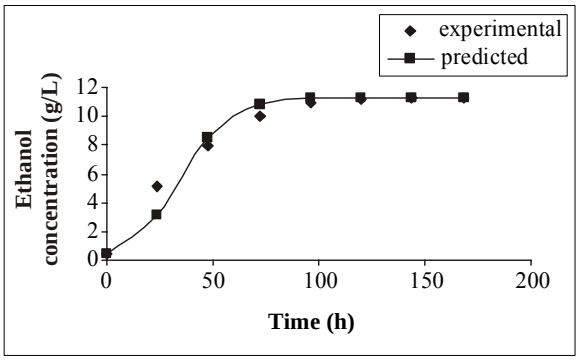

Fig. 5: Comparison on between experimental results and logistic growth model model

CONCLUSIONS

Maximum ethanol concentration was obtained for 0.246 mm size screen in eight days when the substrates were pre-treated with alkali. It was found that the ethanol concentration was higher for bagasse when compared to rice straw. Maximum concentration of 10.59 g/L ethanol

was obtained for bagasse with a substrate concentration of 2% in 0.246 mm size screen. The kinetic data were fitted with logistic growth model and Leudeking-piret model and it was found that the logistic growth model closely represents the fermentation process whereas Leudeking-piret model represents the fermentation process only at the initial stages.

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