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RESEARCH ARTICLE

Production of bioethanol by Solid State Fermentation using paddy straw as a substrate

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Abstract

Depletion of the fossil fuels, global warming alerts and threats are on the rise which leads to the want of alternative fuel sources. Alternative fuel sources like bioethanol and biodiesel should be able to not only fulfill the fuel requirements, but also be able to combat against global warming threats. The best alternative fuel sources are bioenergy in the form of bioethanol and biodiesel. A large amount of cellulose rich substances like paddy straw are generated in tones in India as agriculture wastes. This can be used in the production of bioethanol with the help of microbial catalytic enzyme, cellulase. Ethanol production using solid substrate anaerobic fermentation of paddy straw is attempted and steps are taken to increase the production ratio of ethanol: biomass. *Trichoderma ressei* (MTCC*164) was cultivated using solid substrate fermentation for 65 days and the crude extract was centrifuged, distilled and confirmed by gas chromatography for the presence of ethanol.

INTRODUCTION

In recent years, there has been a widespread resurgence of solid-state fermentation (SSF) process all over the word due to several advantages over systems mainly on engineering aspects[7&10]. One such attempt is made here for conversion of biomass such as paddy straw into fuel.

Paddy straw contains three major components such as cellulose, hemicelluloses and lignin. Accordingly, only cellulose and hemicelluloses has the ability to be converted into sugars[2&5].

The conversion of paddy straw into ethanol includes two major steps: saccharification and fermentation. In the present study, *Trichoderma ressei* (MTCC*164) which can break down the lignocelluloses biomass easily was used. Thereby, pre treatment is skipped which reduced both the cost and time of ethanol production.

MATERIALS REQUIRED

Substrate

Paddy straw was procured from Pother village, Kanchipuram district, Tamil Nadu. It was prepared for solid state fermentation by cutting into approximate pieces of 1 cm.

Microorganisms and inoculum preparation

Trichoderma ressei (MTCC*164) was procured from IMTECH, Punjab, India, which was cultured using protocol prescribed by IMTECH.

Table 1: Composition of Malt extract medium for 1 litre

Ingredients	Quantity
Malt extract	20gm
Peptone	01gm
Dextrose	20gm
Agar powder	15gm

Bioreactor

1litre capped Scott Duran reagent bottles were used as bioreactors. Three reactors were subjected for the present study, in which $3/4^{th}$ (40g) filled with aforesaid substrate. The reactor was moistened with malt extract broth with equivalent weight of substrate. The reactors were inoculated with *Trichoderma ressei* and tightly packed. The set ups were incubated at 25^0C for 65 days.

Table 2: composition of the substrate

Sample	Substrate wt (gm)	Equivalent broth (ml)	Duration in days
SSF1	40	40	65
SSF2	40	40	65
SSF3	40	40	65

Extraction of crude

Sterile muslin cloth is used for filtering the crude enzyme by gently squeezing the sample. The extract was centrifuged at 12,000 rpm for 20 min and the supernatant is separated and collected in 50ml falcon tubes.

Distillation of crude extract

Crude extract was subjected for distillation at 80^oC until the entire extract is evaporated. The distillate was collected in a sterile falcon tube.

Solvent extraction

Ethanol was separated from the distillate from azeotropic water molecules using solvent extraction method using hexane. The top layer consisted of ethanol.

Gas chromatography

To determine the presence of ethanol, Gas chromatography, (GC 1000, Chemito) was done using Porpack Q column.

Column – Porapak Q
Detector-flame ionisation detector (FID)
Oven temperature-150^0 C
Injection temperature-200^0 C
Detection temperature- 200^0 C
Sample injected-1 micro litre
Pressure-4kg/cm^2

Ethanol standards were used as reference.

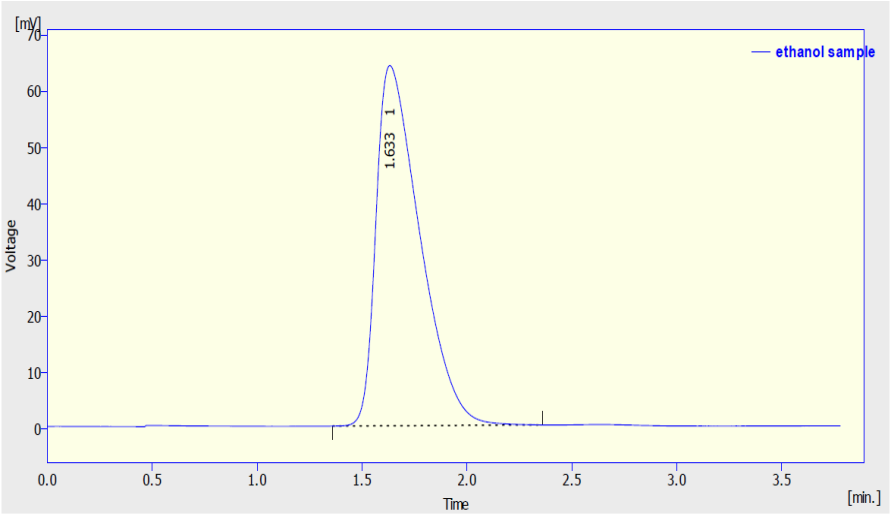
RESULTS

The final sample was used to calculate the percent of ethanol that is produced and calculated to be 16% of the distillate. Therefore the total ethanol yield obtained was 2.8% (v/v) The area of the sample ethanol peak was 928.996. The retention time was 1.633 which was slightly higher than the standard peaks probably due to the meagre presence of other compounds.

Table 3: Yield of Bioethanol

Sample	Yield of crude extract (ml)	Extraction after distillation (ml)	Yield of bioethanol (%)(v/v)	Total yield of Bioethanol (%)(v/v)
SSF1	40	07	16	2.8
SSF2	30	05	15.6	2.7
SSF3	20	03	15.5	2.6

Fig 1: Chromatogram showing sample ethanol peak



Result Table (Uncal - ethanol sample)

	Reten. Time [min]	Start Time [min]	End Time [min]	Start Value [mV]	End Value [mV]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.633	1.360	2.360	0.533	0.745	928.996	64.019	100.0	100.0	0.23
	Total	Total	Total	Total	Total	928.996	64.019	100.0	100.0	

Discussion

The present study had the objective to quantify the amount of ethanol produced and to cut down the cost of production. The major constraint for the successful bioconversion of lignocellulose materials is the physical protection of cellulose by lignin against cellulolytic enzymes. Enzymatic pre treatment is more efficient, though the use of enzymes increases the cost of bioethanol production. But pre treatment was avoided in the present study which reduced both time and cost of bioethanol production.

The most favourable condition for *Trichoderma ressei* to grow is on malt extract medium. It was grown and prepared for fermentation of paddy straw. The gas-pak system maintains anaerobic atmosphere for *Trichoderma ressei* to feed on paddy straw and convert it into ethanol using its cellulolytic enzymes.

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