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## RESEARCH ARTICLE

## Solid Substrate Fermentation of paddy straw for production of biohydrogen using *Trichoderma reesei*

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### Abstract

There is a growing concern arising out of the projection of fossil fuel shortfall towards the mid of 21<sup>st</sup> century. Under this perspective, biological hydrogen production assumes paramount importance as an alternative and renewable energy resource. Biohydrogen is considered as an important key to a sustainable world power supply and is currently being seen as the versatile fuel of the future, with the potential to replace fossil fuels. It has the key prospective to become the ideal means among the range of renewable H<sub>2</sub> production technologies presently existing. Paddy straw is rich of lignocellulosytic materials which can serve as good substrate in solid state fermentation (SSF) to produce the hydrogen. The aim of this study was to explore the possibility of utilizing paddy straw effectively to convert them into fermentable sugars by the production of *in situ* enzyme in SSF, where paddy straw can be used as substrates for the *Trichoderma reesei* (MTCC\*164) fermentation in the production of commercially viable products. In the present study, hydrogen is being produced using SSF of paddy straw and the presence of hydrogen is checked using Gas Chromatography. The maximum yield obtained was 9.9 ml of hydrogen/g of paddy straw. The maximum purity of Hydrogen obtained was 84.5%.

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## INTRODUCTION

From the aspect of energy security and environmental protection, bio hydrogen production from renewable crop straw wastes has been an exciting area of bio-energy production because of its environmental friendly and energy saving process (Boyles, 1984; Elam *et al.*,) By far the majority of study, however, are confined to using pure carbohydrates and carbohydrate-rich waste water (Benemann, 1996; Momirlan & Verziroglu, 2002). The bio-conversion of corn stalk into cellulose-hydrogen is challenging the scientific community because of their complex chemical structures and hard biodegradation. A little information is available on the cellulose-hydrogen production using corn stalk as feedstock so far (Nandi & Sengupta, 1998; Das & Verziroglu, 2001)

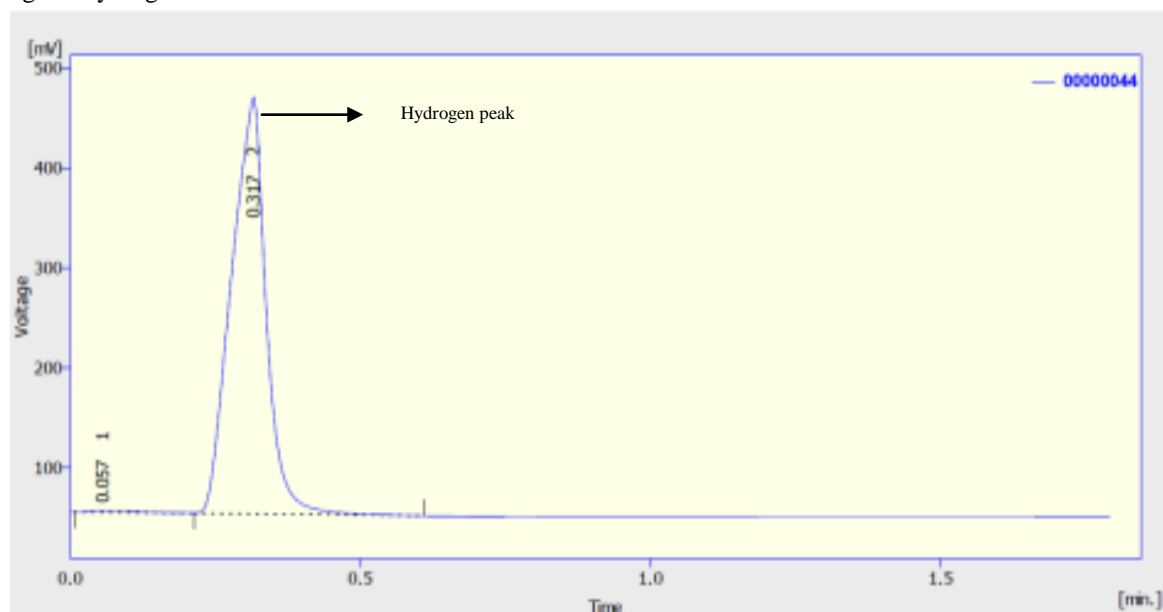
A large amount of cellulose rich substances like paddy straw are generated in tonnes in India as agriculture wastes. This can be used in the production of bioethanol with the help of microbial catalytic enzyme, cellulase (Sandhya *et al.*, 2015). The biodegradation and bioconversion of lignocelluloses into useful products and biological alleviation of pollution from lignocelluloses waste is an enormous environment challenge (Panagiotou *et al.*, 2003). A great variety of fungi can degrade these macromolecules by using a battery of hydrolytic or oxidative process (Perez *et al.*, 2002). The sugars produced can be converted to ethanol, lactic acid and bio hydrogen. For the technique of solid state fermentation (SSF) the operation cost is lower, because simple design machinery and less energy usually are required (Roche *et al.*, 1994). Two cellulases of the filamentous fungus *T. reesei*, cellobiohydrolase II and endoglucanase I are active against non substituted, insoluble cellulose (Bailey *et al.*, 1993).

## Materials and Methods

Paddy straw was procured from Potheri village, Kanchipuram District, Tamil Nadu. *Trichoderma reesei* (MTCC\*164) was procured from IMTECH, Punjab, India, which was cultured using standard protocols prescribed by IMTECH. One litre reagent bottles (schott duran), were used as reactor for SSF for the present study. 50g of paddy straw was filled in every fermentation setup. The reactor was moistened with malt extract broth and was inoculated with *T. reesei*. After 15 days of fermentation a balloon was fastened to the mouth of the reactor to ensure leak proof of gas. The mouth of the reactor was carefully opened and the entire gas was collected in balloon without any escape. A syringe was used to puncture the balloon and the gas thus collected was injected in previously vacuumed storage vials which was corked and sealed. To determine the presence of hydrogen, Gas chromatography (GC 1000, Chemito) was run using Porpack Q column. Standard hydrogen was used as reference.

## Results and Discussion

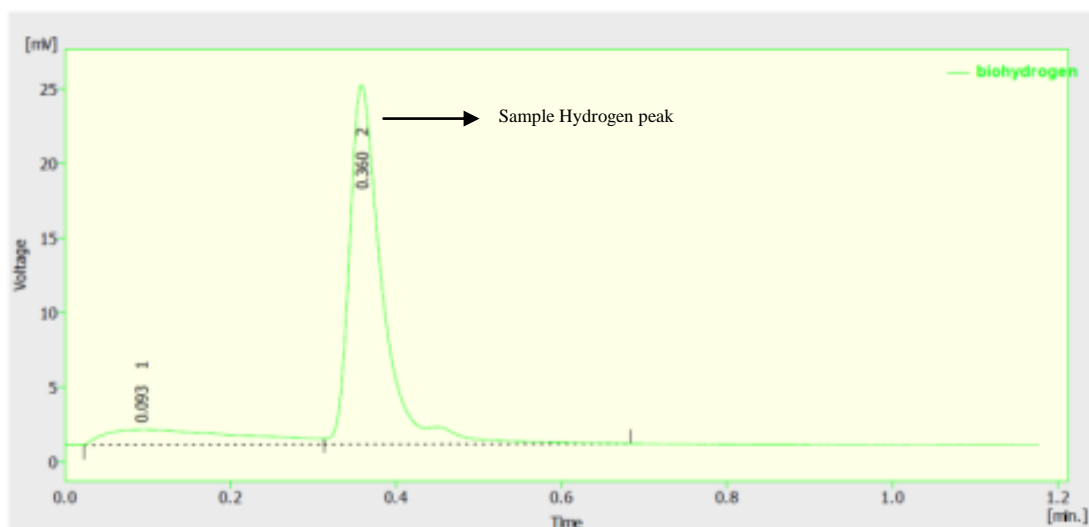
Fig. 1: Hydrogen Standard



Result Table (Uncal - 00000044)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.057	10.146	1.575	0.6	0.4	0.08
2	0.317	1728.570	416.958	99.4	99.6	0.07
	Total	1738.716	418.534	100.0	100.0	

Fig. 2; Graph showing hydrogen peak in sample



Result Table (Uncal - biohydrogen)

	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	0.093	0.023	0.313	1.141	1.187	12.184	1.030	15.5	4.1	0.24
2	0.360	0.313	0.683	1.187	1.245	66.510	24.056	84.5	95.9	0.04
	Total	Total	Total	Total	Total	78.694	25.086	100.0	100.0	

Study shows that wheat straw hydrothermally liberated to a cellulose rich fibre fraction and a hemicelluloses rich liquid fraction (hydrolysate), enzymatic hydrolysis and subsequent fermentation of cellulose yielded 0.41g-ethanol/g-glucose, while dark fermentation of the hydrolysate produced 178.0ml-H<sub>2</sub>/g-sugars ( Prasad kaparajuet al., 2010). In the present study, pre treatment of the paddy straw was avoided, thereby, cutting down the cost and time for the production of bio hydrogen. The net yield of hydrogen from the fermentation setups were relatively lesser when compared to the yield of hydrogen produced from pre treated paddy straw. The minimum yield of hydrogen obtained was 2.1ml of H<sub>2</sub>/g of paddy straw and the maximum yield was 9.9 ml of H<sub>2</sub>/ g of paddy straw. The maximum purity of the bio hydrogen obtained was 84.5%.

Different stages of hydrolysis of the paddy straw were observed. Hydrolysis indicates that *T. reesei* used the substrate and released cellulases into the medium which in turn hydrolysed the lignocellulolytic mass of the paddy straw. The yield of hydrogen increased with more substrate quantity and higher fermentation time.

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