International Journal of Scientific Engineering and Technology
Volume No.6 Issue No.4, pp: 135-137

ISSN:2277-1581
01 April.2017

Biological Pretreatment Technology-A Future Key for Bioethanol Production

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Abstract : Non-renewable fossil fuels and their continuous depletion give rise to the convergence of researchers interest towards renewable and sustainable development of bioethanol production. The present study investigated with the production of ethanol from agricultural waste such as banana pseudo stem and coir pith waste with the help of biological treatment using white rot-fungi like Pleurotus ostreatus and Phanerochaete chrysosporium carried out by SMC (Submerge) and SSC (solid state cultivation). The hydrolysis was done by Aspergillus niger, Aspergillus fumigates and mixed culture of both Aspergillus niger and Aspergillus fumigates. Finally fermentation was done by Saccharmyces cerevisiae. At SSC condition using Phanerochaete chrysosporium gives better result in case of pseudostem.

Key Words- Agriculture, biomass, fermentation, hydrolysis, pretreatment

I . Introduction

Lignocellulosic resources such as paper, cardboard, wood, agricultural residues e.g straws, cobs, nutshells, stalks, bagasse, food industry residues, municipal solid wastes and other fibrous plant material can be used as a energy source. Due to energy source, biomass converting bioethanol production using various methodologies. Biorenewable fuel, referred to as biofuel, is solid, liquid or gaseous fuels that are predominantly produced from biomass **(**Berktay A et .al., 2008; and Konwer D et. al ; 2007**).** Bioethanol is an important product of biotechnology because of the potential to replace some of the liquid fossil fuels in the transportation, to enhance the security of supply and to reduce greenhouse gas emissions **(**Gray K.A et. al; 2006**).** The conversion process includes biomass handling, pretreatment, hydrolysis, fermentation and purification. In general, pretreatment technologies enhance the enzymatic hydrolysis by removing lignin and hemicellulose, swelling pores in the biomass structure, increasing surface area, breaking and regenerating cellulose structure. Yeast is the most commonly used microorganism for ethanol production by fermentation process. Among several genus of yeast that can be used for ethanol production, *Saccharomyces cerevisiae* is most popular, because of its high efficiency in ethanol production, fast growth rates and unique tolerance to environmental stresses such as high ethanol concentration, toxic fermentation inhibitors and low oxygen levels **(**Matsushika, A & Sawayama, S 2008**).** A huge quantity of wastes are generated where it creating various problem in daily life because of straingent regulation of environmental act. The lignocellulosic wastes such as (banana pseudostem and coirpith) and other biomass can be utilized to produce ethanol, which is consider as promising alternative energy source for transport sector. Cellulose linked with B-1,4 glucose units binds tightly with liginin and hemicelluloses forming complex material and hence delignification is a prerequisite step to release cellulose and hemicelluloses from the waste. In recent years effort have been made to produce ethanol from bioconversion of lignocellulosic waste with the help of different microorganism including fungi. The ability of fungi to degrade lignocellulosic materials due o their highly efficient enzymatic system such as enzyme laccase. (Andrew .G et al; 2013; Adenipekun, C.O et.al 2012 **)**. white rot-fungi like ***Pleurotus ostreatus , Phanerochaete chrysosporium*** *and* ***Pleurotus ostreatus*** has the cabability to release cellulose and hemicelluloses from lignocellulosic biomass. A study on the recent developments in key technologies in cellulosic ethanol production has been reported by (Lee *et al*.2008**).**They discussed the various pretreatment techniques based on the composition of lignocelluloses biomass and simultaneous saccharification and co-fermentation for cellulosic ethanol production. The present study investigated with the production of ethanol from agricultural waste such as banana pseudo stem and coir pith waste with the help of biological treatment using white rot-fungi carried out by SMC (Submerge) and solid state (SSC) cultivation.

II. Materials and Methods

Lignocellulosic agricultural waste have been taken for the present investigation **Banana pseudo stem** (*Musa paradisiaca*) and **Coir pith** (*Cocos nucifera)* were collected from local area Sakhigopal, Puri District. The wastes was chipped using lab scale chipper and the impurities were removed by washing with water, drying overnight 60^0c and stored with container for further use.

COIRPITH

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BANANA PSEUDOSTEM

The fungal strain P*leurotus ostreatus (*NCIM-NO-1200) and *Phanerochaete chrysosporium* (NCIM-NO-1197) were collected from National Colletion of Industrial Microorganism, pune, India. Fungal strain were inoculated on Patato dextrose plate (PDA) and incubated for 4-5 days at 35^0 c and finally stored in refrigerator for further use.

Fungal pretreatment of waste

Two strategies SmC (submerged) and SSC (solid state), were applied for pretreatment of lignocellulosic waste. For SmC preatments, 1.5gm of lignocellulosic waste (air dry) was supplemented with 54ml acetate buffer (20mM, pH4.5) plus 1ml spore inoculums to obtain a 5% solid loading. For SSC pretreatments,3gm of were mixed lignocellulosic waste with 14ml acetate buffer(20Mm,pH 4.5) plus 1ml spore inoculums to obtain 75% substrate moisture content(wet basis).Control flask, without fungal inoculation, were prepared along with the pretreatment flask and destructively sampled on 0 and 35 days to quantify the effects of substrate preparation and soaking. Fungal pretreatments were carried out in 250ml conical flasks capped by a silicon stopper with intel and exit lines connected to 0.2 mm filters. The total experiment was carried out with two different fungi like *Pleurotus ostreatus, and p chyrosporim*. Flasks with wastes were autoclaved for 20min (121^0C, 15psi), cooled, mixed with buffer and inoculated with spore suspension. Pretreatment were performed in an air convention incubator at 34◦C for solid sample kept in UV-incubator and submerged sample kept in shaker incubator at 34◦C and flasks were flushed with oxygen for 10min every 3 days starting from day 0.In between flushing with oxygen for 10min every 5 days starting from day 0. In between flushing events, the culture flasks were closed by clamp inlet and exit tubing lines.

Enzymatic hydrolysis and Fermentation

The hydrolysis was done by *Aspergillus niger, Aspergillus fumigates* and mixed culture of both *Aspergillus niger* and *Aspergillus fumigates.* Using 1.5g of biological Pretreated samples were taken in separate 500ml flasks containing 150 ml of buffer (15 mM, $_p$H 5) and then were incubated in a controlled environment incubator shaker (CIS-24 BL, Remi Instruments Ltd., India). The flasks were estimated for the sugar content at 0th hour and after 24 hours by means of DNSA method. After 24 hrs of incubation, one ml of yeast *Saccharamyces cerevisiae* are inoculated in hydrolyzed sample and incubated in a controlled environment incubator shaker (CIS-24 BL, Remi Instruments Ltd., India) 28°C for 72 hours on a shaker at 120rpm (Caputi*et, etal.1968,).* Ethanol was estimated using UV-visible spectrophotometer.

III. Results and Discussion

Percentage of reducing sugar yield after hydrolysis of lignocellulosic waste.

The present study investigated with the Percentage of reducing sugar yield from agricultural waste such as banana pseudo stem and coir pith waste with the help of biological treatment using white rot-fungi like ***Pleurotus ostreatus*** and ***Phanerochaete chrysosporium*** carried out by SMC (Submerge) and solid state (SSC) cultivation. The hydrolysis was done by ***Aspergillus niger, Aspergillus fumigates*** and ***A.niger and A.fumigatus*** represented as **Fig-1 and Fig-2.**

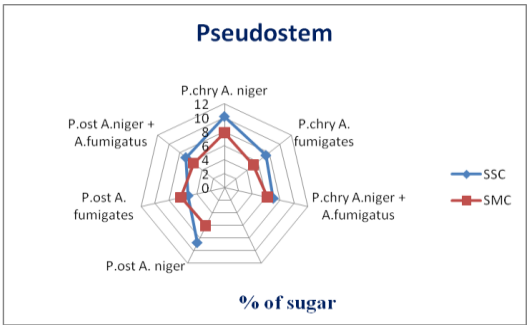

Fig-1 Percentage of reducing sugar yield after hydrolysis of pseudostem

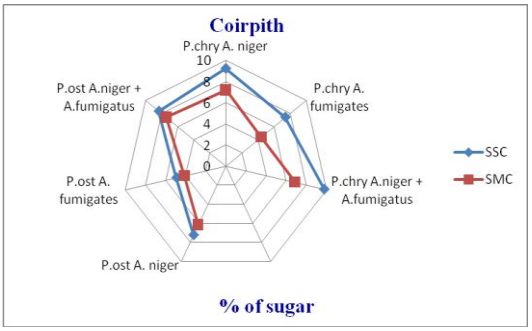

Fig-2 Percentage of reducing sugar yield after hydrolysis of coir pith.

The results showed that sugar yield after enzymatic hydrolysis both in Banana pseudo stem and coir pith using *Aspergillus niger, Aspergillus fumigatus* and *Aspergillus niger and Aspergillus fumigatus* respectively. Among those studies pseudostem using *Phanerochaete chrysosporium* at ssc codition *A.niger* gives better sugar result than others and in case of coirpith *Phanerochaete chrysosporium* at ssc condition mixed culture of both niger and fumigatus better results than others. Phanerochaete chrysosporium has been the model organism for studies of lignin degradation by white rot fungi **(**Kirk TM, et. al 1987**).** Fungi breakdown anaerobically lignin through the use of

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a family of extracellular enzymes collectively termed ‘‘lignases’’ **(**Howard RL et. al; 2003**).** Based on this, biological delignification of wood and paddy straw for ethanol production using Phanerochaete chrysosporium was taken up **.**But, the extent of delignification was insufficient to expose a significant fraction of cellulose for enzymatic hydrolysis.

Percentage of ethanol yield after fermentation

After hydrolysis the fermentation was done by *Saccharomyces cervisiae*. After 72 hours production of ethanol from this microoraganisms. The result showed that both pseudostem and coirpith represented as **Fig 3 and Fig4.**The result showed that banana pseudostem using *Phanerochaete chrysosporium* of *A.niger* , *A.fumigates* and both *A.niger* , *A.fumigates* SSC gives better result than SMC. In case of coir pith using *Phanerochaete chrysosporium* of *A.niger* , *A.fumigates* and both *A.niger* , *A.fumigates* SSC gives better result than SMC. Among those studies both pseudostem and coirpith using *Phanerochaete chrysosporium* at ssc codition *A.niger* gives better result than others.

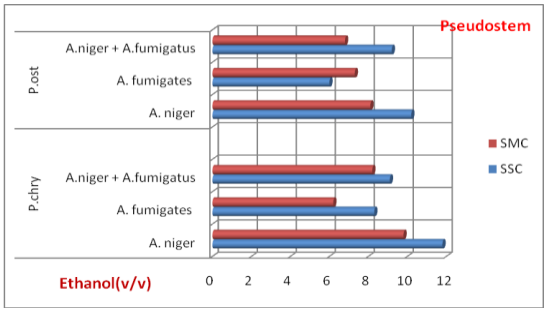

Fig-3 Percentage of Ethanol of Pseudostem

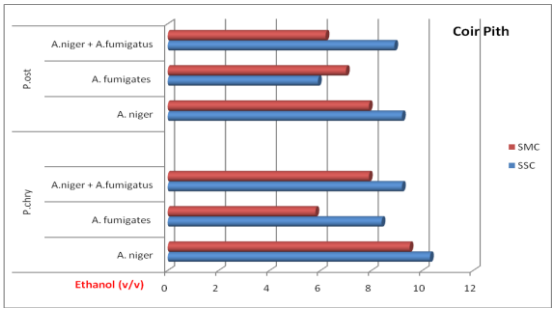

Fig-4 Percentage of Ethanol of Coirpith

(Jian et al. 2008) investigated the potential of microbial pretreatment of cotton stalks by Phanerochaete chrysosporium to degrade lignin and facilitate fuel ethanol production under two culture conditions: submerged cultivation and solid-state cultivation. In this study, the fungal pretreatment of cotton stalks by Phanerochaete chrysosporium showed significant lignin and hemicelluloses degradation when compared with untreated stalks. Recent studies by (Kuhar et al. 2008) have shown that fungal pretreatment of wheat straw for 10 days with a high lignin-degrading and low cellulose producing fungal isolate, RCK-1, resulted in a reduction in acid loading for hydrolysis, an increase in the release of fermentable sugars and a reduction in the concentration of fermentation inhibitors. Ethanol yield and volumetric productivity from RCK-1 treated wheat straw (0.48 g/g and 0.54 g/lh, respectively) were higher than the untreated wheat straw (0.36 g/g and 0.30 g/lh, respectively).

IV. Conclusion

Microbial pretreatment has been previously explored to upgrade lignocellulosic materials for feed and paperapplications. In this case, the microbial pretreatment of trash increased accessibility of sugars for enzymatic hydrolysis **(**Singh P et. al 2008**).**However, the main challenge of fungal pretreatment was found to be the improvement in selectivity for preferential lignin degradation by applying cellulase-deficient or non-cellulose utilizing white rot fungi, thus preserving more cellulose.

References

i. Berktay A., Nas B., 2008, Biogas production and utilization potential of wastewatertreatment sludge. Energy sources Part A, 30, 179-188.

ii. Konwer D., Kataki R., Saikia P., 2007, Pyrolysis of some indigenous tree species of northeast India: effect of pyrolysis temperature and heating rate on the products yieldand char quality. Energy sources Part A, 29, 1433-1442.

iii. Gray K.A., Zhao L., Emptage M., 2006, Bioethanol. Current Opinion Chemical Biology, 10 (2), 141-146.

iv. Matsushika, A & Sawayama, S. (2008). Efficient bioethanol production from xylose by recombinant Saccharomyces cerevisiae requires high activity of xylose reductase and moderate xylulokinase activity. J. Biosci. Bioeng. 106(3): 306-309.

v. Andrew .G & T.Vidal laccase from pycnoporus cinnabarinus & phenolic compound can the efficiency of enzyme mediator for delignifying kenaf pulp be predicted. Bireso.Technol. 131, 536-540.(2013).

vi. Adenipekun, C.O & O.A Okunlade biodegradation of rattan wood & maize stovers by pleurotus ostreatus. Nal. Sci, 10, 49-57. (2012).

vii. Lee, J-S., Parameswaran, B., Lee J-P & Park S-C. (2008). Recent development of key technologies on cellulosic ethanol production, Journal of Scientific and Industrial Research., 67: 865-873.

viii. Kirk TM, Farrell R (1987) Enzymatic ‘‘combustion’’: the microbial degradation of lignin. Annu Rev Microbiol 41:465–505

ix. Howard RL, Abotsi E, Jansen van Rensberg EL, Howard S (2003) Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr J Biotechnol 2:602–619.

x. Jian S, Ratna R, Sharma-Shivappa, Chinn M, Howell N (2008) Effect of microbial pretreatment on enzymatic hydrolysis and fermentation of cotton stalks for ethanol production. Biomass Bioenergy 33:88–96.

xi. Kuhar S, Nair LM, Kuhad RC (2008) Pretreatment of lignocellulosic material with fungi capable of higher lignin degradation and lower carbohydrate degradation improves substrate acid hydrolysis and eventual conversion to ethanol. Can J Microbiol 54:305–313.

***xii.** Singh P, Suman A, Tiwari P, Arya N, Gaur A, Shrivastava AK (2008) Biological pretreatment of sugarcane trash for its conversion to fermentable sugars. World J Microbiol Biotechno l24:667–673.*