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Simultaneous Saccharification and Fermentation of rice bran and ground nut shell for the production of ethanol

William Johnson Arokiasamy*, Vinoth Arulraj Joseph Xavier, Chozhavendhan Sivasankaran, Praveen Kumar Ramanujam, Sathish Alagurajan, Rahul Rajkumar
Anna Bioresearch Foundation, Arunai Engineering College Tiruvannamalai, Tamilnadu.
***Corresponding author: E-Mail: awilliamjoe@gmail.com**

ABSTRACT

Ethanol is the most widely used liquid biofuels. It is an alcohol obtained from fermentation of sugars, starches or from cellulosic biomass. This research aims to develop and assess an enzyme-based biomass-to-ethanol conversion process, which employs agricultural waste materials, as alternative sources of cellulosic material feedstock (rice bran and groundnut shells). Cellulase producing organism *Aspergillus niger* and ethanol producing organism *Saccharomyces cerevisiae* are used in this process to digest the cellulose and to convert them into alcohol. This method implies ethanol conversion in an easy, less expensive method. The cellulosic biomass is pretreated by acid and alkali methods. Simultaneous Saccharification and Fermentation (SSF) process is preferred for this production. The physio chemical parameters (pH and temperature) which influences the production of ethanol were optimized.

KEY WORDS: *Aspergillus niger*, *Saccharomyces cerevisiae*, ethanol.

1. INTRODUCTION

Cellulosic biomass, has been envisaged to be the alternative raw material for bioethanol production, is an ideal source of energy as it is both renewable and existing in large quantities throughout the world. Cellulosic biomass convert in to glucose and other fermentable sugars has been considered in the last few decades, which is an attractive method for ethanol production. However, the production process for the bioethanol from cellulosic materials requires pretreatments such as liquefaction and scarification which is more complicated than its production from sugar or starch-based ones. Lignocellulose is a organic material and is the major structural component of all plants. Due to this organic component Cellulosic biomass are pretreated. Lignocellulose consists of three major components i. Cellulose, ii. Hemicellulose, iii.Lignin. The substrate can be pretreated by chemical methods such as Acid and Alkali. Acid pretreatment involves the use of concentrated or diluted acids to break the rigid structure of the lignocellulosic material. The greater part generally used acid is dilute sulphuric acid (H_2SO_4), which has been commercially used to pre-treat a wide variety of agrobiomass like switch grass, corn stover, spruce (softwood), and poplar. Removal of hemicellulose (acid pretreatment) followed by removal of lignin (alkali pretreatment) has shown to yield relatively pure cellulose. Strong acid allows complete breakdown of components in the biomass to sugars, but also requires large volumes of concentrated sulfuric acid which may result in the production of inhibitory byproduct. Alkaline pretreatment involves the use of hydroxides, such as sodium, potassium, calcium, and ammonium, for the pretreatment of lignocellulosic biomass. The application of an alkali during pretreatment causes the degradation of ester and glycosidic side chains resulting in structural amendment of lignin, cellulose enlargement, partial decrystallization of cellulose and partial solvation of hemicelluloses. Sodium hydroxide has been extensively studied for many years, and it has been shown to disrupt the lignin structure thereby increasing the accessibility of enzymes to cellulose and hemicellulose. We used cellulase producing organism *Aspergillus niger* and ethanol producing organism *Saccharomyces cerevisiae*. Fungi digest the cellulose into monomeric sugars & then the monomeric glucose can then be fermented to ethanol with the help of *S. cerevisiae*. Further, suitable pretreatment is a key step for the effective utilization of cellulosic biomass, due to its intractable nature. Because the cost of raw materials contributes substantially to the cost of ethanol production. The present study aims in the isolation of novel potential thermo tolerant fungus strains& yeast strain from local areas and use them for ethanol production with cellulosic substrate such as rice bran, groundnut shells.

2. MATERIALS& METHODS

2.1 Microorganism: The yeast strain *Saccharomyces cerevisiae* and the fungal strain *Aspergillus niger* were used. Growth media used for *Saccharomyces cerevisiae* is Yeast extract - 3g, Peptone - 10g, Dextrose - 20g, Distilled water - 1 liter, Agar - 15g. The samples were inoculated on to sterile Yeast-extract, Peptone and Dextrose (YEPD) plates. The plates were incubated at 30˚C for 48 hrs. The growth media used for *Aspergillus niger*is PDA - 39g, Distilled water - 1 liter. The culture was plated on PD agar plates and were incubated at 30˚C for 72 hours until the mycelium sporulates black conidia. Inoculum was prepared in 250ml flasks containing 100 ml potato dextrose broth by transferring 2 discs from the PDA plates. The flasks were incubated for 72 hours at 30˚C.

2.2 Substrate: Rice bran &ground nut were used as substrate. The rice bran is the one of the most abundant lingo cellulosic waste ground nut also the abundant cellulosic waste material in the world.

2.3 Location: Rice and ground nut are largely cultivated is the large cultivated crop in Tiruvannamalai district. Fig. 1 and 2 shows the graphical representation of rice bran and groundnut shell production and waste generated by this two crops in last six years.

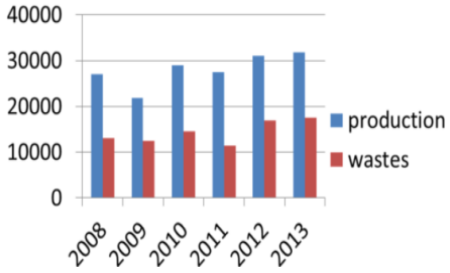

Fig.1.The statistical analysis of rice bran

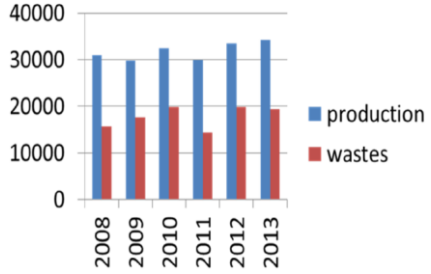

Fig.2.Statistical analysis of ground nut shell

Y axis – In tones; X axis- Years

2.4 Pretreatment methods:

2.4.1 Acid pretreatment: 5 g of substrate was dissolved in 80 ml of 1% HCl and kept in overnight incubation at room temperature. Incubated substrate were sterilized and washed with distilled water to remove the traces of HCl. The sterilized substrates are dried in hot air oven for 24 hours at 100°C to remove the moisture content. This method of acid pretreatment has more disadvantages due to its corrosive nature and they have long residence time.

2.4.2 Alkali treatment: This method of alkali treatment is similar to acid treatment. 5 g of substrate was dissolved in 80 ml of 1% NaOH and kept it overnight incubation room temperature. Incubated substrate were sterilized and washed with distilled water to remove the traces of NaOH. The sterilized substrates are dried at 100˚C in hot air oven for one day to remove the moisture. Alkali pretreatment has more advantages than the acid pretreatment.

2.5 Fermentation: The fermentation is caused by yeast or bacteria which feed on simple sugars. The glucose produced from the hydrolysis described above is fermented with yeast to produce ethanol. Carbon-dioxide is also produced as glucose is consumed. The simplified reaction equation is:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2.$$

The media was autoclaved, cooled down to room temperature and *Aspergillus niger* followed by *Saccharomyces cerevisiae* were aseptically added for fermentative production of cellulosic ethanol. The fermentation was carried out at 30˚C for 120 hrs and the flasks were agitated for 150rpm. 10ml of sample was taken aseptically with sterile micropipette tips. The samples were centrifuged at 4500 rpm at 20mins. After centrifugation the supernatant was collected for distillation.

2.5.1 Simultaneous Saccharification and fermentation process (SSF): The SSF process combines enzymatic hydrolysis of cellulose to a monomer and conversion of monomer to bioethanol. During SSF process, enzymatic hydrolysis and glucose fermentation to bioethanol by baker's yeast proceed concurrently within one vessel. The optimum temperature for SSF was 35^0C. This is a very promising way of producing ethanol due to its capacity to enhance the hydrolysis rate, yield and product concentration when compared to SHF.

Table.1.Requirement for fermentation

Exp no	Rice Bran	Ground nut Shell
1	5g substrate + 250 ml distilled water	5g substrate + 250 ml distilled water
2	Growth medium	Growth medium

Distillation is carried over to produce distilled beverages with higher alcohol content.

2.6 Conformation test: Iodoform test & litmus test were carried out. Formation of yellow precipitate was seen in Iodoform test, change in color of litmus paper from blue to red showed the presence of ethanol.

2.7 optimization: SR exported experimental work on SSF have focused on improving the process by mounting the substrate loading (*i.e*. the content of water insoluble solids, WIS), decreasing enzyme and yeast concentration, and varying temperature and pH.

2.8 pH & Temperature: SSF experiments were carried out at 37°C temperature though the *S.cerevisiae* optimum temperature was around 30°C. The better hydrolysis of cellulolytic enzymes at 55°C, in order to maintain the condition a best suitable compromise at the high end of what *S.cerevisiae* can tolerate is adopted. This condition were carried out simultaneously in acid and alkali pretreated in the respective pH of the substrate.

3. RESULTS&DISCUSSION

3.1 Cellulose recovery: The recovery of cellulose after both the pretreatment methods were quite similar, but cellulose recovery after the acid pretreatment is slightly higher than that of alkali pretreatment. Cellulose concentration after pretreatment with acid is found to be 56% while that after NaOH treatment was 55%. Graph 1 represents the estimation of cellulose from rice bran & Graph 2 represents the estimation of cellulose from ground nut. Graph 3 represents the estimation of ethanol from rice bran & ground nut shell (acid pretreatment). Graph 4 represents the evaluation of ethanol from rice bran & ground nut shell (alkali pretreatment).

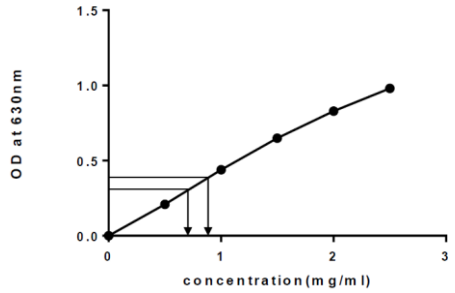

Fig.3.Estimation of cellulose from rice bran

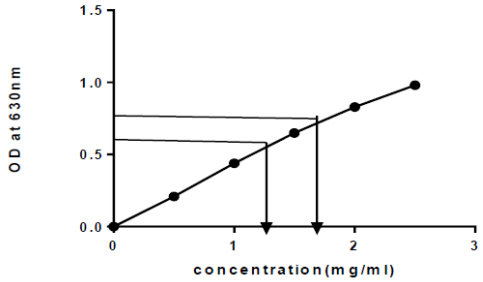

Fig.4. Estimation of cellulose from ground nut shell

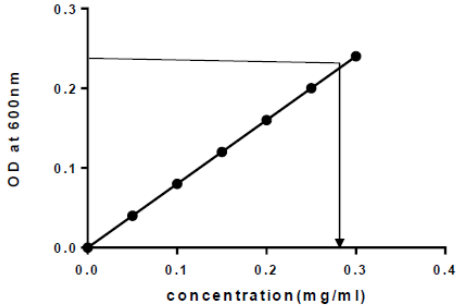

Fig.5. Estimationof ethanol from rice bran & ground nut shell (Acid pretreatment)

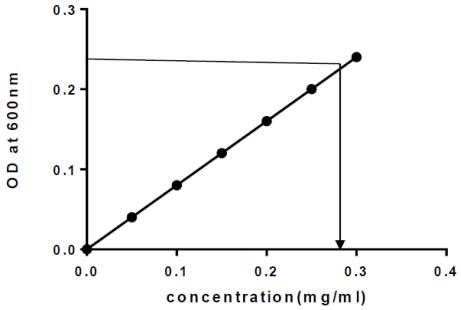

Fig.6.Estimation of ethanol from rice bran & ground nut shell (Alkali pretreatment)

P^H 4 (alkali & acid) shows high yield of ethanol & the temperature at 30^0c shows the maximum yield of ethanol. Graph 5 represents pH 4(acid and alkali). Graph 6 represents the effect of temperature at 30^0c.

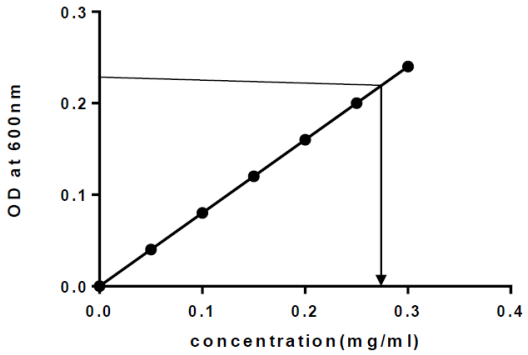

Fig.7. P^H 4 Alkaline

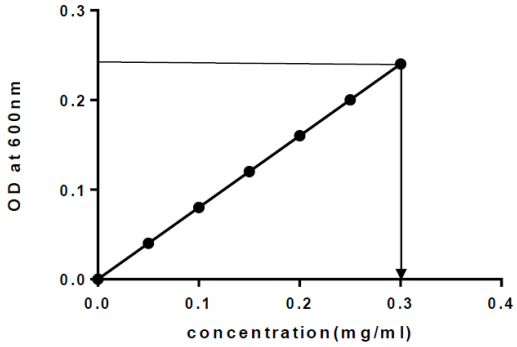

Fig.8. Temperature at 30^OC

In this work, bioethanol production was studied by Saccharification process using *A. Niger* and fermentative production of ethanol by *S. cerevisiae*. The accumulation of cellobiose was observed nearly to 10 g L^{-1} during the initial hours of fermentation, when using the *S.cerevisiae* strain for bioethanol production in SSF process. Both the substrates were treated with 1% NaOH for 2 hrs before enzyme hydrolysis to improve enzyme amenability. Achieved 12.5 g L^{-1} of ethanol using the SSF process with sugarcane bagasse previously acid-pre-treated and NaOH-de lignified, after 48 hrs. of process, corresponding an ethanol efficiency of 32.6%. Comparable results were recorded at the end of SSF process with an ethanol concentration of 12.3 g L^{-1} using the substrate of rice straw pre-treated with alkali and enzymatically hydrolyzed which corresponded to an ethanol efficiency of 40.3%. Normally cellulosic materials can be hydrolyzed chemically or enzymatically. It was found that native biomass is extremely obstinate to enzyme Saccharification, to improve enzyme amenability to lignocellulose fraction a number of study was made to use the fungal culture *A. Niger* as a source of cellulase enzyme in Saccharification step which hydrolyses complex cellulosic substrates by the release of extracellular cellulase enzyme and release simple sugars. Cellulase is an induced enzyme and its production increased with increase in fungal biomass above the incubation period and as simple sugar in the substrate diminished. Temperature has complex effect on enzyme activity and thermal stability of the enzyme. The Saccharification of cellulase enzyme increase with temperature and decline after the optimum temperature is reached which correspondingly increases the kinetic energy of the reaction. The cost of cellulase and recovery of fermentable sugars after enzymatic Saccharification are the vital factors which will decide the tangible cost of biomass to ethanol process .Bioethanol production is a widely studied process for biofuels production. Different workers have studied a variety of raw materials and methods for bioethanol production but, recently it has been observed that lingo cellulosic materials are attracted for its specification in bioethanol production. Hence, we have selected cheaply and abundantly available agro based wastes for bioethanol production. Cellulosic substrates used for bioethanol production by microbial extracellular enzymatic hydrolysis and fermentation yielded 8.9g/l.

Bioethanol was produced enzymatically saccharificing sunflower stalks yielded 0.02g/100g. Enzymatically pre-treated agricultural residues were also used for ethanol production by different fungal cultures.

4. CONCLUSION

We conclude that cellulosic agricultural wastes particularly groundnut shells and rice bran are potential substrates which can be exploited in industries in future for cellulosic ethanol (biofuels) production as they are inexpensive, rich and more significantly renewable. Based on the results the rudimentary cellulosic wastes are used as cheap raw materials. *A.niger* can be used as an intact microorganism for Saccharification of cellulose as a substitute to pure cellulase enzyme, groundnut shells can be utilized and stationary fermentation is ideal method.

REFERENCES

Chaplin M, Bucke C, Effect of pH and ionic strength on enzyme catalysis In: Enzyme technology, Cambridge University Press, 1990.

Cheng YS, Zheng Y, Yu CW, Dooley TM, Jenkins BM, Vander Gheynst JS, Evaluation of high solids alkaline pretreatment of rice straw, Appl Biochem Biotechnol., 162(6), 2010, 1768–1784.

Farrell A.E, Plevin R.J, Turner B.T, Jones A.D, O'Hare M, Kammen D.M, Ethanol can contribute to energy and environmental goals, Science, 311, 2006, 506–508.

Goldstein I, Easter J, An improved process for converting cellulose to ethanol, Tappi J., 28, 1992, 135.

Hernandez-Salas J.M, Villa-Ramirez M.S, Veloz-Rendon J.S, Rivera-Hernandez K.N, Gonzalez-Cesar R.A, Plascencia-Espinosa M.A, and Trejo-Estrada S.R, Comparative hydrolysis and fermentation of sugarcane and agave bagasse, Bioresource Technology, 100 (3), 2009, 1238-1245.

Ibrahim MM, El-Zawawy WK, Abdel-Fattah YR, Soliman NA, Agblevor FA, Comparison of alkaline pulping with steam explosion for glucose production from rice straw, Carbohydr Polym., 83 (2), 2011, 720–726.

Lynd LR, Weimer PJ, Zyl WH, Pretorius IS, Microbial cellulose utilization: fundamentals and biotechnology, MMBR, 66 (3), 2002, 506-577.

Mac Donald DG, Bakhshi N, Mathews JF, Roychowdhury A, Bajpai P, Moo-Young M, Alkaline treatment of corn stover to improve sugar production by enzymatic hydrolysis, Biotechnology Bioeng., 25 (8), 1983, 2067–2076.

McIntosh S, Vancov T, Enhanced enzyme Saccharification of sorghum bicolor straw using dilute alkali pretreatment, Bioresour Technol., 101 (17), 2010, 6718–6727.

Shuler ML, Kargi F, Bioprocess engineering: Basic concepts, 1st ed., Prentice-Hall Inc., New Jersey, 1997, 49-78, 232-239.

Sills DL, Gossett JM, Assessment of commercial hemicellulases for Saccharification of alkaline pretreated perennial biomass, BioresourTechnol., 102 (2), 2011, 1389–1398.

Soto ML, Dominguez H, Nunez MJ, Lema JM, Enzymatic Saccharification of alkali-treated sunflower hulls, BioresourTechnology, 49 (1), 1994, 53–59.

Sukumaran, Rajeev K, Singhania, Reeta Rani, Mathew, Gincy Marina and Pandey, Ashok, Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production, Renewable Energy, 34 (2), 2009, 421- 424.

Taherzadeh MJ, Karimi K, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review, Int J Mol Sci., 9, 2008, 1621–1651.

Takagi M, Abe S, Suzuki S, Emert GH, Yata N, A method for production of alcohol directly from cellulose using cellulose and yeast, Proceedings, Bioconversion Symposium, IIT, Delhi, 1977, 551–571.

Vasquez, Mariana P, Silva, Juliana N.C, Souza, Mauricio B, and Pereira, Nei, Enzymatic hydrolysis optimization to ethanol production by simultaneous Saccharification and fermentation, Applied Biochemistryand Biotechnology, 137-140, 2007, 141-153.

Wingren A, Galbe M, Zacchi G, Techno-economic evaluation of producing ethanol from softwood—a comparison of SSF and SHF and identification of bottlenecks, Biotechnology Progress, 19(4), 2003, 1109–1117.

Zhao Y, Wang Y, Zhu JY, Ragauskas A, Deng Y, Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature, Biotechnology and Bioengi., 99 (6), 2008, 1320–1328.