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Pretreatment of Rice Straw for Bio-ethanol Production: A Review.

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ABSTRACT

Bioethanol is considered as one of the most likely next generation fuel for automobiles because it is neutral in carbon content and can be produced from the renewable resources like the lignocellulosic biomass that is obtained from rice which is a major by-product of agriculture and is produced in a large amount in India. There are many technological barriers like pretreatment, hydrolysis and fermentation of the reducible sugars which are needed for efficient conversion of bioethanol from lignocellulosic biomass. In this review various pretreatment processes used for the extraction of bioethanol and resolving the technological challenges to develop a low-cost as well as the efficient commercial process.

Keywords: Bioethanol, Lignocellulose, Pretreatment, Lignin.

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INTRODUCTION

The inescapable reduction of petroleum supply from the world and the enlarging greenhouse effect has increased the demand of nonpetroleum source of energy. Use of ethanol has reduced carbon dioxide emission from the atmosphere [1]. Production of ethanol from the cellulosic material has the solution for some of the recent problem of environment, economic and energy that the world is facing today [2]. Bioethanol has been recognized as the most propitious renewable source of energy, especially as a transport fuel. It is a resource that does not add CO₂ to the atmosphere but on combustion it releases volatile organic compound, nitrogen oxide and carbon monoxide in low concentration [3]. Rice straw can be used for the bioethanol production as it is one of the largest available biomass feedstock in the world and has about 90% annual global production. Being one of the staple crops of the world's population and having annual global production of about 465.078 MT. [4]. Lignocellulose that is present in the rice straw has a very complex structure as it mainly comprises of cellulose (35-50%), hemicellulose (25-30%), and lignin (25-30%) [5]. It is resistant towards degradation because of the presence of lignin which negatively affects the conversion step and limits the ethanol production.[6] [2]. Cellulose being the major component of the plants cell wall is a glucan polysaccharide which has a large source of energy affording great potential to convert into biofuel [2]. There are three main steps for the extraction of bioethanol from the raw material first is the pretreatment where lignin is removed and converted to monomeric sugar that is pentose and hexose. Second is the hydrolysis which mainly engages in clearing of the polymers of cellulose and hemicelluloses using certain enzymes for producing glucose monomer [7]. Finally third is fermentation process that is used for the conversion of glucose to bioethanol [8].

Physical Pretreatment

Milling Pretreatment

In the milling pretreatment rice straw was grinded and put into Erlenmeyer flask. Then it was moistened using distilled water. Further it was incubated for about 2 hours and he finally it was mixed so that reducing sugar can be extracted. The yield of Total reducing sugar after milling pretreatment was 1.44 g/L [3].

Milling and autoclaving pretreatment

According to Biswas the rice straw was dried using the forced-air oven at 55°C for about 24 h and then passed through hammer mill for milling to reduce the size to 1.27 mm. This milled rice straw (15% w/v) was then mixed with 1% v/v H₂SO₄ and treated in autoclave at 121°C for 1h. There was about 35% conversion of the cellulose present in the rice straw to reducible sugar [9]. The grinded rice straw was put into Erlenmeyer flask, moistened and then treated with stem using autoclave at temperature of 121°C and 1.5 bar pressure for about 20 min. The yield of total reducing sugar was found to be 6.35 g/L at the end to this process [3].

Milling and Gamma γ irradiation pretreatment

In the pretreatment done by milling and irradiation, grinded rice straw was given various doses (50 and 70 Mrad) of this γ radiation. Then this radiated rice was put in Erlenmeyer flask and further moistened with distilled water. After incubation of 2 h the sugar is extracted by filtration. 6.62 g/L was found to be the yield of the Total reducing sugar at the end of this pretreatment [3]

Milling, γ irradiation and autoclaving pretreatment

In the combination of milling, irradiation and autoclaving grinded rice straw was given γ radiation of various frequency and after moistened with distilled water put into Erlenmeyer flask, then autoclaved at 121°C and 15 bars for 20 min. After autoclaving the content was extracted. The yield of reducing sugar was found to be 5.03 g/L at the end of this pretreatment [3].

Ultrasonic pretreatment

According to the studied conducted by the Ultrasonic wave can also be used to remove the lignin content of the rice straw. The waves were used for 10, 20, 30, 40, 50, and 60 min at 250 W, 40 KHz. [10]

Chemical Pretreatment

Sodium chlorite and sodium hydroxide treatment

In the chemical treatment that was done using sodium chlorite and sodium hydroxide powdered rice straw was dried and was treated with sodium hydroxide (1-5%) concentration and sodium chlorite (5%) concentration. Further it was washed with deionised water several times then dried by using hot air oven for about 24 hrs at 70°C. The cake was then processed for microbial saccharification. Maximum yield was obtained with 5% NaOH and 5% NaCl [2]

Alkaline hydrogen peroxide

In the treatment that was done using hydrogen peroxide rice straw was grinded and then treated with 2.5 % concentration of alkaline hydrogen peroxide (NaOH + H₂O₂) which is at a pH of 4.5 [11]. 20 gm of rice straw was taken after cutting and then suspended 160 ml of 1% NaOH aqueous solution. Then it was kept for boiling for 15 min to 2 h. Further the residue was collected and washed with tap water so that the pH is neutralized and then dried. The cellulose and carbohydrate yield at the end of the treatment was found to be 99± 0.4% and 80± 0.6% respectively after 70 min [12]. In the study done by Cheng were he used NaOH by first dissolving it in DI water and then incubating at 55°C and then the solution is mixed with the rice straw [13]

Physio-chemical pretreatment

Phosphoric acid pretreatment

In the phosphoric acid pretreatment 50g of dried material was taken and mixed well with 400 ml of concentrated phosphoric acid. It was then incubated in rotary air bath at 120 rpm and temperature of 50°C for about 1h. After the reaction has occurred the solution was poured in 1.2L of pre-cold acetone and then mixed. Further the mixture is centrifuged at 8000 rpm for 10 min, the supernatant is taken and suspended in 1.2 L of acetone and then centrifuged three times. The residue was again washed with distilled water and centrifuged three times. During the last step the pH was adjusted to 5.0-6.0 with the help of 10M NaOH and then finally the pretreated material was collected [1].

Aqueous-ammonia soaking treatment

In the pretreatment that was one using aqueous-ammonia soaking method 10 g of rice was soaked in the aqueous-ammonia solution. The solids were then separated from the solution by using filtration cloth and it was washed with 2L of distilled water till the pH of the solution reached 6.5-7.0. Further dried in vacuum-drying-oven at 45°C for 3 days [14].

Sulfuric Acid Pretreatment

In the sulfuric acid treatment the rice straw was grinded to about 833 µm in size and then 600g was soaked in 4l of 0.5% sulfuric acid solution for about 20h. This mixture was added into 10l reactor, where it was steam heated for 1.5 min till 15bar pressure is achieved. Then this pressure is remained for 10 min. Further the solution is cooled within 3 min to achieve 2 bar pressure, the material is collected and washed five times with tap water and finally filtered [8].

Sodium hydroxide pretreatment

During the sodium hydroxide treatment the rice straw was grinded and dried in the hot-air oven at 70°C. This dried rice was treated by 1% sodium hydroxide (NaOH) at solid-to-liquid ratio of 10% (w/v). The residue is collected by filtration and then washed with distilled water so that the pH can be neutralized [15]. According to pretreatment performed by Wei powdered rice straw was taken and first treated with dilute sulfuric acid (0.25~1.5% v/v) at a temperature of (100~160°C) for about 10 min to 1 h. then the slurry was filtered and the filter cake was collected and suspended in a solution of dilute sodium hydroxide and placed in autoclave with a pressure ranging from 0.0 to 1.0 Mpa at 120~160°C. For the neutralization the residue was washed with tap water. The yield of reducing sugar obtained after the pretreatment was 46% [16].

Wet air oxidation pretreatment

Wet Air Oxidation Reactor of 1.8 L volume was taken in the wet air oxidation treatment method and 30 g or dried rice straw mixed with 500 g of water and 1 g of Na_2CO_3 was added in the WHO reactor. The suspension was then mixed and sealed so that there is no leakage. Pressure of the air (at 0.5 and 1.0 MPa, corresponding to 0.05 and 0.11 mol of O_2 respectively) was applied and then the solution was heated. During heating the temperature was kept $\pm 5^\circ\text{C}$ and a constant stirring of 100 rpm. The suspension was then left for the reaction to occur and finally the pretreated slurry was cooled and filtrated, giving a cake that is rice in cellulose and hemicelluloses [6].

Pretreatment using PCS (peptone cellulose solution) medium

In the PCS pretreatment method 5g of the sample was taken in a 200 ml of flask which contain 100 ml of autoclaved PCS medium (0.1% yeast extract, 0.5% peptone, 0.2% CaCO_3 , 0.5% NaCl, 0.5% filter paper, pH 7.0). This culture was the incubated at 50°C under the optimum conditions. Once the paper strip was degraded and rice straw had become soft, 5 ml of culture is transferred into fresh enrichment medium. This process was then repeated 10 times. The remaining culture as stored and kept in cold [5].

Microwave/ alkali pretreatment

Domestic microwave oven was used with the microwave frequency was 2450 MHz. 20 g of rice straw grinded and then suspended in 160 ml of 1% NaOH aqueous solution in a beaker and then it was placed at the center of rotating circular glass plate inside the microwave oven and the microwave treatment was given for 15 min to 2 h. Yield of about $99 \pm 0.6\%$ and $75 \pm 1.2\%$ for cellulose and carbohydrate respectively was found [12].

Lime pretreatment

The rice straw according to Saha was milled and 15.0 % w/v was taken along with 1.5 % w/v of lime and slurry was made. The slurry was further autoclaved at 121°C for 1 h. The pH of the pretreated rice straw was then adjusted to 5.0 using concentrated solution of HCl. The yield of the reducing sugar was increased with the increased doses of lime and found that at 100mg lime g⁻¹ the yield was 126 ± 1 mg [17].

AFEX (Ammonia Fiber Explosion) treatment

In AFEX pretreatment liquid ammonia was given to 1-2 kg of biomass at a temperature of 90°C [18][19]. 75% of the glucose was released after 24 h of hydrolysis [20]. Ethanol has a yield of about 83% at the end of the pretreatment [21]

Biological Pretreatment

Was carried out with fungal, *Trichoderma reesei* [2]. Different fungal strain spores such as F66, F94 or F98 (F94 and F98 were strains of *Trichoderma viride* and *Aspergillus terreus* respectively) can also be used by inoculating them in the grinded rice husk and incubating them for 7 days at a temperature of 30°C [3]. Studies that have been conducted show that white-rot fungi are the most effective microorganism used for the pretreatment of lignocellulose that is present in agricultural waste [22]. Many fungi produce hydrogen peroxide with the help of certain enzymes like glyoxal oxidase, ary- alcohol oxidase and pyranose-2 oxidase which degrade the lignin content of the rice straw [23].

CONCLUSION

Bioethanol serves as the best alternative for the renewable energy that can be extracted from agricultural waste. There as many hindrance that occur during the extraction process, this is due to the complex structure of the lignocellulose content of the biomass. Various pretreatment processes are used for the removal of this lignin content and the exposure of the glucose so that it can be hydrolyzed and fertilized to yield bioethanol. The pretreatment processes may be physical, chemical, biological or a combination of physical and chemical treatments. It was found that out of the different physical pretreatment performed the

combination or milling, γ -irradiation and autoclaving has a better yield. In chemical pretreatment alkaline hydrolysis was found to have higher yield as compared to other chemicals. Therefore, a combination to the two physio-chemical pretreatment was used, and there was comparatively higher yield. Biological pretreatment was also done with help of some fungal species. The main aim is to find out the most cost-effective and high yielding treatment process.

REFERENCES

- [1] Li Hui, Kim Nag-jong, Jiang Min, Kang Won Jong Chang Nam Ho, Simultaneous saccharification and fermentation of lignocellulosic residues pretreated with phosphoric acid–acetone for bioethanol production. *Science direct* 100 (2009) 3245–3251
- [2] Srivastava Ajeet Kumar, Agrawal Pushpa, Rahiman Abdul, Delignification of Rice Husk and Production of Bioethanol. *International Journal of Innovative Research in Science, Engineering and Technology* ISSN: 2319-8753
- [3] Abo-State Marvate A., Ragab Ahmed M.E., EL-Gendy Nour sh., Farahat Laila A. and Madian Hekmat R., Bioethanol Production from Rice Straw Enzymatically Saccharified by Fungal Isolates, *Trichoderma viride* F94 and *Aspergillus terreus* F98. *Soft*, 2014, 3, 19-29
- [4] Potumarthi et al., Simultaneous pretreatment and saccharification of rice husk by *Phanerochete chrysosporium* for improved production of reducing sugars. *Science direct* 128 (2013) 113–117
- [5] Wang et al., Characterization of a microbial consortium capable of degrading lignocelluloses. *ScienceDirect*, 102 (2011) 9321–9324
- [6] Banerjee et al., Evaluation of wet air oxidation as a pretreatment strategy for bioethanol production from rice husk and process optimization. *Science Direct*, 33 (2009) 1680–1686
- [7] Wongwilaiwan et al., Analysis of a thermophilic lignocellulose degrading microbial consortium and multi-species lignocellulolytic enzyme system. *Science direct* 47 (2010) 283–290
- [8] Karimi et al., Ethanol production from dilute-acid pretreated rice straw by simultaneous saccharification and fermentation with *Mucor indicus*, *Rhizopus oryzae*, and *Saccharomyces cerevisiae*. *Science Direct* 40 (2006) 138–144
- [9] Biswas et al., Process for obtaining cellulose acetate from agricultural by-products. *Science Direct Carbohydrate Polymers* 64 (2006) 134–137
- [10] Yu et al., 2009 Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull, *Bioresource Technology* 100 (2009) 903–908
- [11] Krishna et al., Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast, *Bioresource Technology* 77 (2001) 193-196
- [12] Zhu et al., Pretreatment by microwave/alkali of rice straw and its enzymic hydrolysis, *Process Biochemistry* 40 (2005) 3082–3086
- [13] Cheng et al., Evaluation of High Solids Alkaline Pretreatment of Rice Straw, *Appl Biochem Biotechnol* (2010) 162:1768–1784
- [14] Kyong Ko et al., Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes, *Bioresource Technology* 100 (2009) 4374–4380
- [15] Sandhu et al., Two-Stage Statistical Medium Optimization for Augmented Cellulase Production via Solid-State Fermentation by Newly Isolated *Aspergillus niger* HN-1 and Application of Crude Cellulase Consortium in Hydrolysis of Rice Straw, *J. Agric. Food Chem.* 2013, 61, 12653–12661
- [16] Wei et al., Pretreatment and Saccharification of Rice Hulls for the Production of Fermentable Sugars, *Biotechnology and Bioprocess Engineering* 2009, 14: 828-834 DOI/10.1007/s12257-009-0029-8
- [17] Saha et al., Lime pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol, *Science Direct biomass and bioenergy* 32 (2008) 971 – 977
- [18] Chundawat SP, Venkatesh B, Dale BE (.2007). Effect of particle size based separation of milled corn stover on AFEX pretreatment and enzymatic digestibility. *Biotechnol. Bioeng.* 96(2): 219-31.
- [19] Thomsen MC, Belinda A (2007). Wet oxidation pretreatment of lignocellulosic residues of sugarcane, rice, cassava and peanuts for ethanol production. *J. Chem. Technol. Biotechnol.* 82(2): 174-181. doi: 10.1002/jctb.1648.
- [20] Sun Y, Cheng J (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresour. Technol.* 83(1): 1-11. doi: 10.1016/S0960-8524(01)00212-7.
- [21] Jeoh T, Agblevor FA (2001). Characterization and fermentation of steam exploded cotton gin waste. *Biomass and Bioenergy* 21(2): 109-120. doi:10.1016/S0961-9534(01)00028-9.



- [22] Akin DE, Rigsby LL, Sethuraman A, Morrison WH, Gamble GR, Eriksson KEL (1995) Alterations in structure, chemistry, and biodegradability of grass lignocellulose treated with the white rot fungi *Ceriporiopsis sub Vermispora* and *Cyathus stercoreus*. *Appl Environ Microbiol* 61:1591–1598
- [23] Martinez AT, Ruiz-Duenas FJ, Martinez MJ, Del Rio JC, Gutierrez A (2009) Enzymatic delignification of plant cell wall: from nature to mill. *Curr Opin Biotechnol* 20(3):348–357