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Review Article

POTENTIAL OF ETHANOL PRODUCTION FROM COTTON STALK: A REVIEW

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ABSTRACT

Cotton stalk, a byproduct of cotton production is abundantly available lignocellulosic biomass, and has enormous potential as replacement for fossil fuel. Due to recalcitrant nature of lignocellulosic biomass, direct conversion without pretreatment always results in an extremely low yield. Therefore, prior to ethanol fermentation, the feedstock needs to be processed by pretreatment and saccharification technology, in order to retain maximum fermentable sugars. In this regard, various efforts have been taken by the researchers from all around the world, to develop a cheap, efficient, and environmental friendly pretreatment technique. This paper aims to present a comprehensive review on outcomes of some extensive investigations in the laboratories on ethanol production potential from cotton stalk.

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INTRODUCTION

Cotton is an important commercial crop playing a key role in terms of human subsistence and industrial raw material. The global cotton production during 2016-17 has been estimated as 23.68 million metric tons. India is emerging as the largest cotton producer in the world, estimated cotton production is about 5.88 million metric tons, and contributes to 26% of the world cotton production. It has the distinction of having the largest area under cotton cultivation in the world ranging between 10.9 million hectares to 12.8 million hectares and constituting about 38% to 41% of the world area under cotton cultivation (CCI, 2017). India has the unique distinction of being the only country in the world to cultivate all four cultivable *Gossypium* species including *Gossypium arboreum* and *herbaceum* (Asian cotton), *Gossypium barbadense* (Egyptian cotton) and *Gossypium hirsutum* (American Upland cotton) (Binod *et al.*, 2012). After harvesting the cotton bolls, the entire plant consists of stalk and leaves, which remain in the field. The use of cotton stalk as firewood for household energy needs or burned on the ground, causing serious environmental pollution and biomass waste. It has been reported that India generated 18.9 million metric tons of cotton plant waste, out of which, 7.4 million metric tons residue is used by farmers themselves as firewood for household energy needs and remaining 11.4 million metric tons residues do not have proper use. Moreover, it cannot be

used as fodder for animals. Because of its lignocellulosic nature, cotton stalk has the potential to be used as a renewable raw material for a variety of commercial applications, such as the production of ethanol, glucose, xylose, xylitol, etc. (Kaur *et al.*, 2012).

Compositional analysis of cotton stalk

The major chemical composition of cotton stalk is cellulose, hemicellulose and lignin but their concentration varies depending on growing location, season, harvesting methods, as well as analysis procedures (Agblevor *et al.*, 2003). Silverstein *et al.*, (2007) reported that the key component of cotton stalk was found to contain 41.8% Holocelluloses (in which 31.1% glucan, 8.3% xylan, 1.3% arabinan, and 1.1% galactan was detected), acid insoluble and soluble lignin were found to contain 27.9 and 2.2 % respectively. Binod *et al.*, (2012) showed that the cotton stalk collected from Andhra Pradesh (India) contains 33.3% glucans and 14.8% xylan along with very small proportions of arabinan and mannan, while galactan was not detected by them. The analysis conducted by Baig, (2014) indicated that the debarked cotton stalk was found to contain 65.32% Holocelluloses (in which 42.40% glucan and 23.20% xylan was detected) and 24.18% lignin, while moisture and ash were found to be 3.05% and 0.95% respectively. These observations showed that, in cotton stalk,

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glucan is the dominant polysaccharide and xylan is identified as the second most abundant sugar.

Outcomes of various research works carried out for conversion of cotton stalk to ethanol

Although numerous pretreatment methods exist, each one has its own advantages and disadvantages. Various pretreatments are better suited for specific feed stocks (Alvira *et al.*, 2010). In this section we discuss the efforts taken by different researchers to develop efficient pretreatment technique, which includes biological, chemical, mechanical and thermal process, as well as their combinations, to speed substrate hydrolysis.

Silverstein *et al.*, (2007) investigated the effect of ozone, in comparison with sodium hydroxide and sulfuric acid on cotton stalk and found good results by using sodium hydroxide solution for pretreatment as compare to ozone and acid, and reported that 2% (w/v) concentration of sodium hydroxide at 121°C for 90 minutes was found to be an optimum process for delignification, and upon enzyme hydrolysis, it gives highest cellulose conversion of 60.8%. During same year, Chen *et al.*, (2007) reported the potential of using ensiling as cost effective pretreatment for bioethanol production from agricultural residues such as cotton stalk and wheat straw, and concluded that, unlike different methods of pretreatment; it is highly time consuming technology. However, Shi *et al.*, (2009) investigated the effect of *Phanerochaete chrysosporium*, as biological pretreatment on cotton stalk under submerged cultivation (SmC) and solid state cultivation (SSC) and found significant lignin degradation i.e. 19.38% and 35.53% for SmC and SSC respectively. Further, Binod *et al.*, (2012) optimized the process of alkaline pretreatment in high pressure reactor equipped with pitch blade turbine stirrer, followed by enzymatic hydrolysis using cellulase. The resultant data showed that 4%NaOH at 180°C for 45 minute with mixing substrate at 100 rpm is an optimum strategy for maximum delignification process, and the hydrolysis efficiency of pretreated cotton wastes material recorded as 96%, while the process efficiency based on glucose recovery was 53% (based on cellulose to glucose conversion). Moreover, Kaur *et al.* (2012), achieved 46.6% of lignin degradation using 4% alkali treatment at 121°C for 60 minute, increase 83.2% of glucan content, compared with undertreated biomass. This is followed by enzymatic hydrolysis using combination of 20 filter paper cellulose unit, 45 IU of β glucosidase, and 15 IU of pectinase, per gram of dried substrate for 48 hours, resulted in 42.29 g/L of glucose and 6.82 g/L of xylose, furthermore when it was fermented using *pichiakudriavzevii* HOP-1, consumed about 99 % of glucose in 24 hours and produced an ethanol concentration of 19.82 g/L. The work carried out by Baig and Dharmadhikari, (2012) showed that 2% NaOH for 60 minutes at 121°C was suitable for maximum delignification process which removed 0.201 gram of lignin per gram of biomass. Enzyme unit of 100 CMC per gram of pretreated (delignified) biomass was found to be optimum concentration for hydrolysis, which yielded total sugar of 0.49 g/g of biomass, corresponds to a concentration of 24.5 g/L. Furthermore, when it goes to fermentation using co-culture of *Saccharomyces cerevisiae* and *Pachysolen tannophilus*, utilized 97.81% of total available sugar and gives an ethanol concentration of 9.56 g/L, corresponds to a fermentation efficiency of 76.85%. The yield of ethanol was recorded as 0.191 g/g of biomass, 0.298 g/g of

holocelluloses and 0.392 g/g of fermentable sugar, while cell mass concentration in fermentation was recorded as 12.20 g/L (Baig and Dharmadhikari, 2014).

Du *et al.*, (2013) performed the high pressure assisted alkali pretreatment of cotton stalk and reported that, maximum cellulose content of pretreated cotton stalk residue was achieved as 64.07%, by treating it with 3% NaOH with solid liquid ratio of 20:1, at pressure of 130 kPa, for 40 minutes. Upon hydrolysis it gives maximum cellulose conversion of 45.82% and yielded reducing sugar of 0.293 g/g of biomass. Jiang *et al.*, (2015), exposed different parts of cotton stalk to liquid hot water pretreatment and found that, cotton boll shell and cotton stalk obtained after pretreatment was with weight loss of 46.93% and 38.85% respectively, and upon fermentation it gives an ethanol yield of 0.21g/g, and 0.18g/g of biomass respectively. In addition to that, Wang *et al.*, (2016) studied the effects of various methods, i.e. dilute sulfuric acid pretreatment (DSAP), ultrasound assisted alkali pretreatment (UAAP), and high pressure-assisted alkali pretreatment (HPAP), on enzymatic hydrolysis and ethanol fermentation they reported that, HPAP led to the highest reducing sugar and ethanol yields (271.70 mg g⁻¹ and 45.53%, respectively) compared with UAAP and DSAP. The resent study performed by Christopher *et al.*, (2017), showed that, 2.5% alkali pretreatment of cotton stalk effectively delignified the biomass, as results 80% hydrolytic efficiency achieved, and upon fermentation, it gives theoretical maximum efficiency of 76%. In another study, Singh *et al.*, (2017) treated cotton stalk with 0.15 mol/L FeCl₃ for 20 min, as pretreatment and received an ethanol concentration of 9.8 g/L, corresponds to a yield of 0.37g/g of sugar consumed.

DISCUSSION

Pretreatment step is referred to as the technological bottleneck for ethanol production from lignocellulosic wastes. It is an important tool for cellulose conversion processes and is essential to change the structure of cellulosic biomass to make it more available to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier *et al.* 2005). However, in case of untreated biomass, during enzymatic hydrolysis, cellulases components, including β -glucosidase and endoglucanase have more binding affinity towards lignin than to the carbohydrates, resulting in lower efficiency of saccharification. Hence, to achieve maximum hydrolysis of cellulosic biomass, which is prerequisite for ethanol fermentation, an appropriate delignification treatment is required (Gupta *et al.*, 2009).

From the data, it was noticed that, much research has done on alkaline pretreatment, as compare to acid and biological pretreatment, and researchers tried to prove that alkaline pretreatment followed by enzyme hydrolysis is more economical and environmental friendly compared to the other pretreatment methods. The major effect of alkali pretreatment is the saponification of intermolecular ester bonds which crosslink lignin and carbohydrates, thus increasing porosity and internal surface of the biomass matrix as well as decreasing the degree of crystallinity of cellulose, resulting in improved susceptibility of remaining polysaccharides to enzyme attach during hydrolysis (Sun and Cheng, 2002). Furthermore, alkali will remove the acetyl and uronic acid groups from

hemicellulose to enhance the accessibility of enzyme (Ramirez, 2005). The remaining lignin rich residues recovered from the alkaline wash can be used as feed stock for generating electricity and steam (Hamelinck *et al.*, 2005). Alkaline pretreatment process utilizes lower temperature and pressure compare to other pretreatment technologies (Balat *et al.*, 2008). However, unlike acid pretreatment, it is much more time consuming (Mosier *et al.*, 2005) and some of the alkali is converted to irrecoverable salt or incorporated as salt into the biomass during reaction (Silverstein, 2004). Beside sodium hydroxide, calcium hydroxide (lime) is also an effective pretreatment agent which is the least expensive chemical with safe handling among all hydroxides. Furthermore, calcium can be recovered from the reaction system by introducing carbon dioxide for calcium hydroxide regeneration (Karr and Holtzapple, 2000).

Next to alkaline pretreatment, is acid pretreatment, which is further categorizes as concentrated acid and dilute acid treatment. Concentrated acid process provides complete and rapid conversion of cellulose to glucose and hemicellulose to 5-carbon sugar with little degradation, but the critical factor is needed to make the process economically viable by optimizing sugar recovery and recovery of acid for recycling. The primary advantage of the concentrated acid process is the potential for high sugar recovery efficiency, but this process offers more potential for cost reductions than the dilute sulfuric acid process (Demirbas, 2007). Dilute-acid hydrolysis is a cheap and fast process to obtain sugar from lignocellulosic biomass; however, a significance drawback of dilute-acid hydrolysis is the generation of several by-products during the process, some of them is toxic to fermenting microorganism (Palmqvist and Hahn-Hagerdal, 2000).

Pretreatment of lignocellulosic biomass can also be carried out by microbial degradation of lignin. Lignin is degraded by different classes of enzymes, which are produced by different microorganism, such as white-rot fungi like *Pleurotostreatus* and *Pycnopouscinnabarinus* etc. Because of the lignin's complexity and random phenylpropanoic polymeric structure, enzymes engaged in its decomposition have to exhibit broad substrate specificity. For efficient degradation, and due to the high occurrence of carbon-carbon type bonds, oxidases and peroxidases are preferred over hydrolases (Janusz, *et al.*, 2013). Therefore, these organism produces some combinations like, lignin peroxidase (LiP) and manganese peroxidase (MnP), fungi producing MnP and laccase, while some other produces LiP and laccase, and fungi which produce neither LiP nor MnP, but laccase and aryl alcohol oxidase or some other enzymes. Fungal laccases are multi-copper phenol oxidases that oxidize numerous phenolic compounds and aromatic amines using molecular oxygen as a terminal electrons acceptor (Giardina, *et al.*, 2010; Janusz, *et al.*, 2013). Compared to physical and chemical process, biological pretreatment is more complicated and time consuming. These technologies could greatly simplify pretreatment, but yields are low and little experience with these approaches exists. One main challenge of this pretreatment is to preserving cellulose from fungal culture and purified without loss of sugars.

CONCLUSION

Great potential of cotton stalk as feedstock for ethanol production; and outcomes of the research about its utilization, showed that the technology is still at laboratory or pilot level, and to overcoming societies emerging fuel demand, continuity of this work is necessary for the development of techno economical feasible method at commercial level. In addition to that, less efforts towards biological pretreatment was observed and for this, isolation of potential laccase and cellulase producers and there scale up for biological pretreatment is necessary.

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