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Optimization of Detoxification with Over Liming and Charcoal Treatment for Increasing the Fermentability of Cotton Stalk Hydrolyzate

KEYWORDS: cotton stalk, acid hydrolyzate, detoxification, over liming, charcoal

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ABSTRACT *Fermentability of lignocellulosic hydrolyzate decreases by retarding microbial fermentation due to presence of inhibitors released during hydrolysis. These inhibitors can be removed by applying proper detoxification process. Among different process, present study focuses on detoxification of cotton stalk acid hydrolyzate by over liming followed by activated charcoal treatment for removal of sugar byproducts i.e. furans (furfural and 5, hydroxy methyl furfural) and traces of lignin in the form of phenolic compounds for the purpose to increase the fermentability of hydrolyzate. In the present investigation, optimization was carried out by overliming up to various pH ranges from 7 to 12 while charcoal concentration was used from 1% to 5% percent. Finally, optimum concentration was achieved by overliming with pH 10 and 4% activated charcoal which causes 92.69% and 88.89% reduction in furans and phenolics respectively with 19.84% decrease in fermentable sugar concentration was observed.*

INTRODUCTION

Fast depletion of conventional energy resources and increasing energy demands diverts the concentration of research towards an alternative energy sources which must be renewable and environmental friendly.Among various processes, ethanol from lignocellulosic biomass is promising method of alternative energy generation. The lignocellulosic biomass include, trees, shrubs, yard waste, wood product, grasses and agricultural residues such as wheat straw, corn stover, rice straw, cotton stalk etc.(Silversteinet al., 2007). Ethanol production from this agricultural biomass requires series of treatment include pretreatment, hydrolysis and fermentation(Balatet al., 2008).Hydrolysis of biomass by sulfuric acid is well known method to obtain fermentable sugars.However, hydrolyzate obtained contains not only fermentable sugars but also some furans such as furfural and 5-hydroxymethyl furfural which are formed by degradation of sugars and various phenolic compounds. These compounds in the hydrolyzate inhibit the fermentation of sugars by microorganisms. Therefore, for achieving high fermentability detoxification of hydrolyzate is necessary before the fermentation to remove inhibitors(Palmqvist& Hahn-Hagerkal, 2000).Various methods have been studied for improving the fermentability of hydrolyzate including enzyme treament, overliming, evaporation, extraction with organic solivent, steam stripping, ion exchange, activated carbon treatment etc(Miyafugiet al., 2003).

In the present study optimization of over liming and charcoal treatment has been carried out on acid hydrolyzate of cotton stalk, which is one of the abundantly available cropresidues in India for the purpose to remove maximum inhibitory compounds and to increase the fermentability of hydrolyzate.

MATERIAL AND METHODS

Collection of raw material

The cotton stalk was collected from farmer's field in Marathwada region of Maharashtra state, India

Physical pre-treatment of biomass

The cotton stalk was shredded and bailed in the field and was debarked, chopped, dried and ground to pass 1-2 mm sieve in laboratory. Dried sample was stored in sealed plastic bags at room temperature until further use.

Compositional analysis

A major portion of biomass feedstock is made up of carbohydrates, which are polysaccharide in nature and primarily composed of glucose, xylose and arabinose subunits while another major portion is lignin. These sub units (glucose, xylose and arabinose) were quantified by HPLC (Zodiac. Ltd) using laboratory analytical proceure-002(LAP-002) standard protocol of NREL(Ruiz andEhrman, 1994).The lignin was also determined as per NREL procedure.

Acid hydrolysis

Acid hydrolysis was carried out in two stages including decrystallization of biomass with 75% H_2SO_4 (Merk Sp. Gr 1.84) at fixed sample acid ratio of 1:2 (by weight) till the color of the paste turned brown without resulting into the oxidation by acid in the first stage followed by dilution of hydrolyzate up to 1 N with distilled water. Finally the hydrolyzate was treated with steam at 121°C for 30 minutes and heated up to four hour at 90°C in water bath (Baig and Dharmadhikari, 2012).

Neutralization and detoxification

After acid hydrolysis, the hydrolyzate was detoxified by over liming and activated charcoal treatment(SrilekhaYadavet al., 2011).

Optimization of over liming

The optimization of over liming was carried out by increasing pH from 7.0 to 12.0 separately using calcium oxide (CaO) and keeping it for an hour. The slurry was then filtered to remove precipitation followed by centrifugation (3000g, 20minutes) to remove traces of salt precipitation. Later the pH of hydrolyzate was brought back to pH 6 using dilute H_2SO_4(Martinezet al., 2000).

Optimization of activated charcoal treatment

In order to obtain the optimization of charcoal treatment, increasing concentration of activated charcoal was added to hydrolyzate from 1% to 5% (w/v) separately along with stirring for half an hour followed by filtration through vacuum filter (Geet al., 2011).

Analytical methods

Total reducing sugars

After appropriate dilution the solubilisation of fermentable sugars were determined by DNS (3, 5-dinitrosalicyclic acid) method of Miller (1959).

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D- Glucose
Glucose concentration, obtained after every treatment was determined by enzymatic method of glucose oxidase and peroxidase based on Bergmeyer's methods (1972) of enzymatic analysis.

Phenolic compounds
Total phenolic estimation of hydrolyzate was carried out by Folin-Ciocalteu methods (Singleton and Rossi, 1965).

Furans
The bi-product of sugars i.e.furfural and 5-hydroxy methyl furfural was determined the method given by Martinez et al. (2000).

Statistical analysis
Statistical analysis were carried out in factorial completely randomized design (CRD) by software MAUSTAT developed by department of statistics ofVasantraoNaikMarathwada Agriculture University,Parbhani,Maharashtra, India.

RESULTS AND DISCUSSION

Compositional analysis of cotton stalk
The major chemical composition of cotton stalk is cellulose, hemicellulose and lignin but their concentration varied depending on growing location, harvesting methods as well as analysis procedure(Agblevoret al., 2003). Silverteinet al. (2007) from United States found 30% cellulose, 13% hemicellulose and 31% lignin while Ververisetal. (2004) (from Grees) found 40% α-cellulose and 17% lignin. The studies conducted by Binodet al. (2012) showed that the cotton stalk (Gossypiumhirsutum) collected from India (Andhra Pradesh) contains 33.3% glycan and 14.8% xylan along with very small proportion of arabinan and manan.

Cotton stalk (Gossypiumhirsutum) used in this study collected from Marathwada region (India) was composed of 42% glycan and 22% xylan while other ingredient of hemicellulose was in very small proportion. The lignin content was 24.18%. Comparatively high amount of sugars and less amount of lignin was noted in present study as compare to different reports which was might be due to debarking, pretreatment; as was observed by John harkins and John Row (1971),who reported that 40% to 55% of total lignin is only present in bark of soft wood.The amount of lignin present in herbaceous plant material ranges from 26% to 34% and specifically the amount of lignin in the bark of soft wood was higher than expected.

Acid hydrolysis
Acid hydrolysis of biomass was carried out by using H_2SO_4 in two stages including concentrated acid decrystallization followed by dilution up to 1N along with steam at 121°C and heating at 90°C for four hours as was done in previous studies. Hydrolysis process releases0.494g of sugar per gram of biomass and specifically dextrose concentration was 0.363g/g of biomass along with fermentation inhibitors such as furans (1.971mg/lit) and phenolic (4.9g/lit). Presence of this mixture is toxic to microorganisms used in fermentation studies and required proper neutralization and detoxification as next step before fermentation.

Neutralization and Detoxification
Fermentation using undetoxified hydrolysate is characterized by slow kinetics, with limited yield and productivity. This is due to presence of a variety of compounds which acts as inhibitors to the microbial metabolism. To overcome this problem, lignocellulosic hydrolysate need to be neutralized from these inhibitors, thereby becoming more suitable for fermentative microorganism.

In the present study optimization of over liming and charcoal treatment has been carried out on acid hydrolysate of cotton stalk, for the purpose to remove maximum inhibitory compounds and to increase the fermentability of hydrolysate. In order to study the dynamic behavior of detoxification of dilute acid hydrolysate, combination of over liming and charcoal treatment were applied subsequently and obtained results are summarized in table 1.and2. respectively.

Effect of over liming on hydrolysate
Detoxification by over liming is an effective method for removal of inhibitors like furans (furfural and hydroxymethyl furfural). Review reveals that, increasing the pH up to 9-10 with lime and readjustment to 5.5 with H_2SO_4 is an effective method but the optimum concentration of lime varies and depends on type and concentration of acid in hydrolysate (Martinez et al., 2000).

For the purpose to optimize lime addition, various concentration of hydrolysate (based on pH) has prepared by adding dried lime, made it from neutrality (pH-7) to alkalinity (pH-11). The results are summarized in Table 1.and Fig. 1. shows that, quantified decrease in the concentration of inhibitors like furans were observed by increasing the pH up to 10 which gives maximum reduction in furans i.e. 1.971 mg/L to 0.312 mg/L (84.17% reduction) while comparatively less amount of phenolics were reduced from 4.90 g/L to 4.18 g/L (14.68% reduction). Total sugars losses were also being reported to the tune of statistical significance which reduced from 0.494 g/g of biomass to 0.422 g/g of biomass (14.58% reduction) and specifically glucose was reduced up to 10.6%. It was noticed that over liming has drastically affects the furans reduction while other inhibitors are comparatively less affected by it as was already observed by Martinez et al., (2000). Above pH 10 the reduction in furans was not observed up to the level of statistical significance but the amount of sugars was decreased more fluently as compare to previous treatment. Therefore by increasing pH up to 10 is considered as optimum lime concentration for detoxification of acid hydrolysate of cotton stalk for getting maximum fermentable sugars.

Effect of activated charcoal
Over liming was not dynamically affected on phenolics as compare to furans there for after over liming, efforts were taken to remove phenolic compounds by exposing it with activated charcoal. Being good adsorbent, activated charcoal treatment can effectively remove phonic compounds from hydrolysate. The amount of phenolic compound in present hydrolysate was higher than expected (4.9 g/L) as compared to previous reports in literature, which might be due to avoiding separate chemical pretreatment for delignification. There for optimization of charcoal treatment on cotton stalk hydrolysate after over liming and neutralization were carried out by varying their concentration from 1% to 5% as shown in Table 2.and Fig.2.

From the data it was demonstrated that 4% charcoal treatment was an efficient concentration for maximum reduction in phenolic compounds. The total phenolic were removed from 4.181 g/L to 0.545 g/L (86.97% reduction), nearly same results were observed by Geet al., (2011); where they removed 96.6% phenolics by using activated charcoal from hydrolysate. However comparatively fewer amount of furans were also reduced, which ranged from 0.312 mg/L to 0.144 mg/L. No doubt some amount of sugars were also loss during treatment which ranged from 0.422 g/g of biomass to 0.396 g/g of biomass (6.17% reduction) and specifically glucose was reduced up to 5.48%., which were also reported by Miyafugiet al., (2003) and Martinzet al., (2001).

CONCLUSION

Conclusively, the optimized detoxification studies of cotton stalk hydrolysate (1 N strength) was achieved by overliming up to pH 10 and keep it an hour followed by filtration and brought back to pH 6 using H_2SO_4, later on 4% charcoal treatment for half an hour followed by filtration gives maximum reduction in inhibitors including 92.69% furans and 88.89% phenolics while 19.84% total fermentable sugar (in which 15.49% glucose was present) losses were also be re-

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ported during the process, as shown in Table 3.

Table 1. The concentration of sugars, furans and phenolics treated with various liming strength.

Liming with different pH	Fermentable sugar (g/g of biomass)	Glucose (g/g of biomass)	Total furans (mg/lit)	Total phenolics (g/lit)
Acid hydrolyzate	0.494	0.368	1.971	4.900
pH-07	0.461	0.353	1.105	4.772
pH-08	0.453	0.351	0.889	4.636
pH-09	0.435	0.340	0.576	4.318
pH-10	0.422	0.329	0.312	4.181
pH-11	0.404	0.312	0.312	4.090
pH-12	0.369	0.273	0.288	4.090
SEm±	0.013	0.007	0.257	0.297
C.D. at 5%	0.041	0.023	0.781	0.900

Table 2. Concentration of sugars, furans and phenolics treated with various charcoal concentrations.

Charcoal treatment	Fermentable sugar (g/g of biomass)	Glucose (g/g of biomass)	Total furans (mg/lit)	Total phenolics (g/lit)
Lime treated	0.422	0.329	0.312	4.181
1% charcoal	0.416	0.325	0.288	2.909
2% charcoal	0.411	0.316	0.240	1.409
3% charcoal	0402	0.313	0.168	0.863
4% charcoal	0.396	0.311	0.144	0.545
5% charcoal	0.369	0.281	0.144	0.500
SEm±	0.015	0.010	0.021	0.257
C.D. at 5%	0.046	0.032	0.067	0.793

Table 3. Concentration of sugars, furans and phenolics after optimized detoxification treatment

Analytical Solution	Total reducing sugars	Glucose	Furans	Phenolics
Acid hydrolyzate	0.494g/g of biomass	0.363g/g of biomass	1.971mg/lit	4.909g/lit
Over limed solution	0.422g/g of biomass	0.32g/g of biomass	0.312mg/lit	4.181g/lit
Charcoal treated	0.396g/g of biomass	0.311g/g of biomass	0.144mg/lit	0.545g/lit

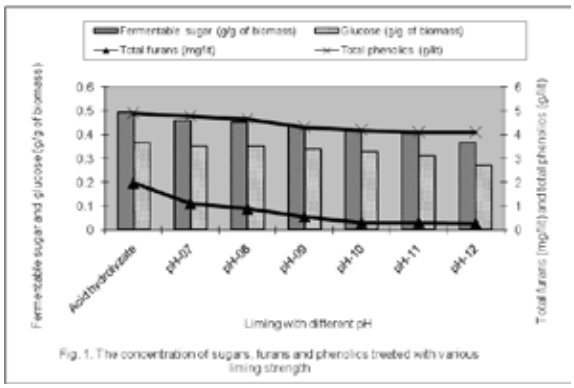

Fig. 1. The concentration of sugars, furans and phenolics treated with various liming strength

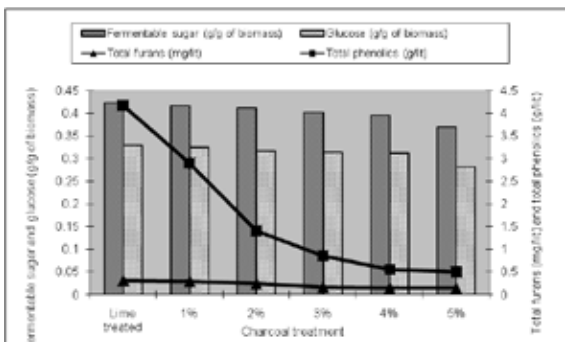

Fig. 2. Concentration of sugars, furans and phenolics treated with various charcoal concentrations

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