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Original Research Article

Bioethanol production from wheat straw using minimal acid use applying acid hydrolysis

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Abstract

Selecting the raw material and efficient, cost effective utilization are the key factors in lignocellulosic biofuel production. In this study an agro waste plant was used to acid hydrolyse by minimal use of sulphuric acid (0.7%) and physical extraction of hemicelluloses hydrolysate by autoclave for different time periods. The hydrolysate thus obtained on breakdown of its oligosaccharide and over-liming gives significantly improveed fermentable sugar, pentose. Fermentation for bioethanol from this hemicellulosic acid hydrolysate by *S. stipitis* NCIM3507 under semi-anaerobic condition was evaluated. The selected acid hydrolysis conditions and detoxification yields hydrolysate fermentable to ethanol.

Key Words: Wheat straw, hemicellulose, acid hydrolysis, bioethanol.

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INTRODUCTION

Among liquid transportation fuel, anhydrous ethanol (99.7%) serves as a good alternative fuel to petroleum as it has similar attributes like density, melting point, boiling point, vapour pressure, pKa, pKb, dipole movement, cetane number, flashpoint and octane number[1]. Since ethanol has 35% oxygen that results in complete combustion due to which bioethanol gains the immense importance. Bioethanol production from natural biomass adds no net carbon dioxide to atmosphere as it is again converted to biomass[2] (Figure 1). Many starch crops are available for bioethanol production but this first generation biofuel causes food versus fuel competition[3]. Lignocellulosic biomass has no such problem as it comes from waste part of the crops or from wasteland plants. Lignocellulose is renewable source and found globally in large quantities[4].

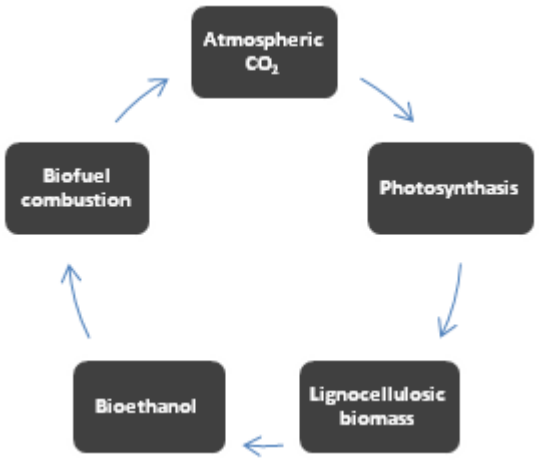


Figure 1:

Cell wall part of plant biomass has 38% to 50% of cellulose which is homopolymer of D-glucose. Cellulose microfibrils are filled by amorphous hemicellulose which is a heteropolymer of pentose sugar (xylose, arabinose) and some hexose sugars. Lignin is biopolymer of phenylpropane derivatives like P- hydroxy phenyl, syringyl and guiacyl monomeric unit which act as cement material on cellulose framework. This layer of lignin

prevents the microbial degradation of plant cell wall[5,6] Thus, the removal of hemicelluloses and lignin is compulsory for full economic saccharification of cellulose. Removed hemicellulose has 23 to 30% of total cell wall sugars. So its utilisation is equally important for industrial bioethanol production. The industrial bioethanol production involves various steps which are summarised in Figure 2. Pretreatment is the key step of bioethanol production which removes lignin in the form of phenolics, separate out hemicellulosic sugars in hydrolysate and exposes cellulose microfilaments for saccharification by cellulase activity. Pretreatment step increases the sugar yield from 20% without treatment to 90% with treatment[7]. Acid hydrolysis is the most accepted pretreatment method; but like all other pretreatment procedures it also produces sugar and lignin derived fermentation inhibitors. Dilute acid pretreatment and alkali pretreatment are relatively inexpensive[8] but both are coupled with thermal hydrolysis process like microwave treatment or pressure cum heat treatment of autoclave[9]. Wheat is only second largest producing crop after rics and whose yield reached about 730million tons in 2014 (1,2). This clearly indicates vast mass of left-out straw which may be important feedsock for biofuel whilst in the want of proper technology it is generally burned in the field causing great impact on economic waste and air pollution. WS is a typical lignocellulosic biomass that mainly comprises cellulose, hemicellulose, and lignin. Cellulose and hemicellulose could be hydrolyzed into monomeric sugars such as glucose, xylose, and arabinose, which could then be converted to biofuels such as bioethanol and methane[10]. *Scheffersomyces stipitis* is a well-known pentose sugar fermenting yeast. It can utilize both pentose and hexose sugar of hydrolysate of various pretreatment origin to ethanol. The present work was undertaken to optimize the production of hemicellulosic hydrolysate from an agro waste i.e. wheat straw by impregnating it in acid or alkali followed by hydrolysis through autoclave under pressure. Obtained hemicellulose hydrolysate was examined for fermentation to ethanol after detoxification with lime using *S. stipitis*.

MATERIALS AND METHODS

Source material and its processing: Agriculture waste wheat straw was collected from a local farmer. It was milled with particle size about 1mm, dried in pre dried container in convection oven maintaining the temperature at 45 ± 3°C for 48 h and stored at -20°C in a polyethylene bag according to NREL, LAP, 2008 protocol[11]. To calculate the percent of total solids, following equation was used:

$$\%T_{45} = \left(\frac{(W_f - W_t)}{(W_i - W_t)}\right) \times 100$$

Where: % T_{45} = percent total solids of a sample oven dried at 45ºC, W_t = tare weight of freeze-drier container, W_i = initial weight of container and sample W_f = final weight of container and sample.

Acid and alkaline impregnation: Dried material (10 g) was poured into 250 ml Erlenmeyer flasks separately followed by 100 ml of 0.7% (v/v) sulphuric acid (H_2SO_4) and incubated for 4 hour [12]. The surplus acid was decanted after impregnation.

Hydrolysis: The prehydrolysate flasks were autoclaved at 121°C at 15psi for 15 and 30 min in two sets of experiments.

Breaking of oligosaccharide Hemicellulose hydrolysate obtained after pretreatments (pH 1.3) was subjected to heating in boiling water bath at 100°C for 10 min. The amounts of total sugars and pentose released were measured. Distilled water was added to make up any loss of volume in hydrolysate.

Detoxification: Acid hydrolysate with an initial pH of about 1·5 was subjected to detoxification with lime (over-liming) until the pH was raised to 11 [13].The mixture was heated for 10 min followed by neutralisation to pH 5.6 by adding 0.1 N hydrochloric acid. Insoluble residues were removed by filtration using whatman No. 4, and the filtrate (treated hydrolysate) was collected for further use as fermentable sugars.

Microorganism and its Maintenance: *Scheffersomyces stipitis* (formerly *P. stipitis*) NCIM 3507 was incurred from National Collection of Industrial Microorganism (NCIM), NCL, Pune. Culture was maintained on agar slant containing (g l^{-1}): xylose, 20; yeast extract, 3; malt extract, 3; peptone, 5; and agar, 20. The medium used for inoculum preparation contained (g l^{-1}): D-xylose, 50; glucose, 5; yeast extract, 3; malt extract, 3; peptone 5; pH 5. To prepare the inoculum, a 250-ml Erlenmeyer flask containing 50 ml medium was inoculated with yeast from a fresh agar slant, and incubated at 30°C on a rotary shaker at 150 rpm for 48 h prior to use. 5 ml aliquot of this culture was transferred to 100 ml fermentation medium.

Fermentation: Hydrolysate was supplemented 30% by fermentation medium [13] with (gL-1): Xylose, 20; yeast extract, 3; peptone, 5; KH_2PO_4, 2; $(NH_4)_2SO4$, 1; MgSO4.7H2O, 0.5; trace element solution, 1 ml/l; the pH adjusted to 5.0. The trace element solution contained (gL-1): $CuSO_4.5H_2O$, 2.5; $FeCl_3.6H2O$, 2.7; $MnSO_4.H_2O$, 1.69; $Na_2MoO_4.2H_2O$, 2.42; $ZnSO_4.7H2O$, 2.87; $CoCl_2.6H_2O$, 2.38; and H_2SO_4 (conc.) 3 drops. A negative control of unsupplemented hydrolysate medium and positive control of fermentation medium alone were kept.

The media were inoculated as above at 100 rpm and 32 °C temperature for 6 days for aerobic fermentation on a shaker incubator.

Distillation method: After fermentation the medium was subjected to distillation. The flask containing fermentation medium was kept in water bath at 100 °C and distillation was continued till 50 ml of the distillate was obtained.

Calculation of total solids: Oven dry weight (ODW) of the sample, using the average total solids content was determined by the LAP, NREL standard method for the determination of total solids in biomass[14].

$$ODW = \frac{Weight_{\ air\ dry\ sample}\ x\,\%\,Total\ solids}{100}$$

Where,

OWD = the weight of biomass mathematically corrected for the amount of moisture present in the sample at the time of weighing; Weight $_{air\ dry\ samples}$ = Weight of sample air dried.

Analytical Methods: Assay of total reducing sugar was carried out by DNS (dinitrosalycilate) method[15] pentose sugar by Orcinol reagent method[16]. Ethanol was quantified in the distillate applying Potassium dichromate reagent method[17]. Calculation of amount of acid soluble lignin (ASL) was done by NREL, LAP method using following formula[14].

$$\%\ ASL = \frac{UVabs\ \ x\,Volume_{\ filtrate}\,x\,Dilution}{\varepsilon x ODW_{sample} \times Pathlength}\ x\ 100$$

Where; UV*abs* = average UV-Vis absorbance for the sample at appropriate wavelength; ε = Absorptivity of biomass at specific wavelength; ODW_{sample} = weight of sample in milligrams; Pathlength = path length of UV-Vis cell in cm

Statistical Control: All experiments were executed in triplicates and three times. Statistical significance of the means was evaluated using MS-Excel statistical tool, one-way analysis of variance. Subsequent comparisons were performed using the least significant difference (LSD) test. Differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

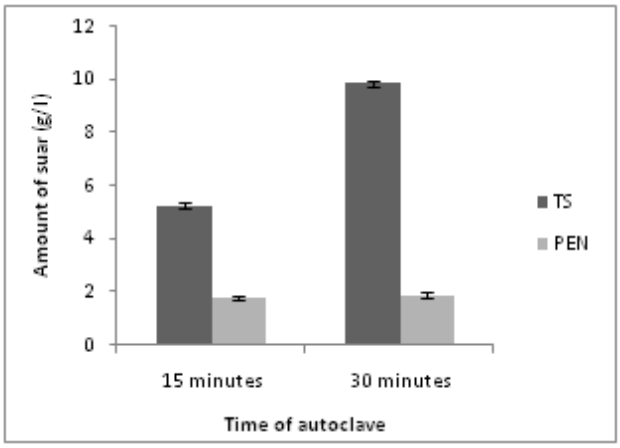
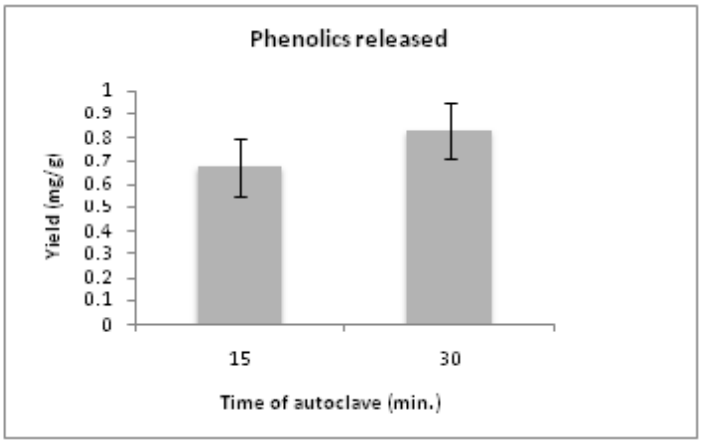


Figure 2:



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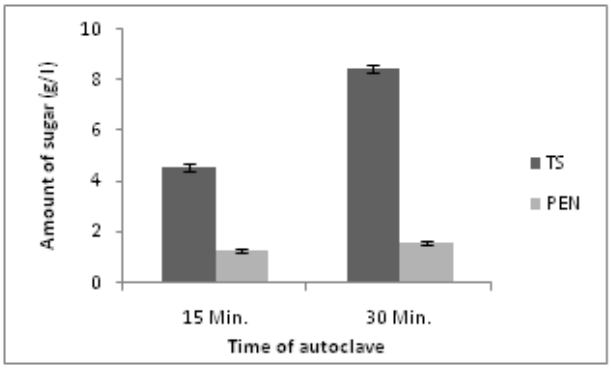

Figure 4:

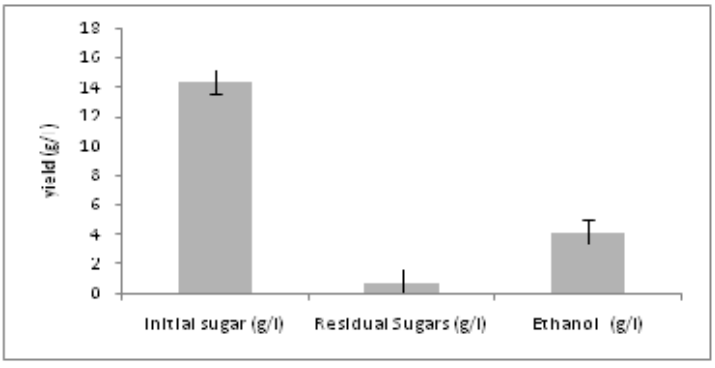

Figure 5:

Figure 2: Release of sugars by wheat straw as a result of different heating periods by autoclave (TS-total sugar; PEN-pentoses)

Figure 3: Release of sugars by wheat straw as a result of different heating periods during autoclave followed by overliming
Figure 4: Acid soluble lignin (ASL) in the terms of phenolics released during acid hydrolysis of wheat straw
Figure 5: Sugar utilization and ethanol production during fermentation of detoxified wheat straw hemicelluloses fermentation by *S. stipitis*.

Feedstock: The feedstock used in this study is agricultural waste i.e. wheat straw, abundantly available worldwide and is already being used[18].

Single step hydrolysis of wheat straw: Oven dried biomass have total dry content/ percent total solid of 78% in wheat straw (in this case having less than 10% moisture content), similar to the earlier studies[19]. This dried biomass was that taken for further studies.

Effect of acid hydrolysis on wheat straw: As a result of standard pretreatment and dilute acid hydrolysis with acid concentration (0.7% sulphuric acid) reported to be optimum for this analysis with two different time of resilient heat and pressure. The increase in the amount of sugar released in hemicellulose hydrolysate may be due to breakdown of cellulosic hexose sugars. The amount of sugar released after oligosaccharide degradation without lime treatment (detoxification) in 15 minute autoclave was 4.56 g/l (total sugar) and 1.3 g/l (pentose sugar). After 30 minute autoclave total sugar released were 8.46 g/l, pentoses were 1.52g/l. **(Figure 2)** On extending this experiment, sugar released after detoxification and oligosaccharide-breakdown increases considerably viz., 5.2 g/l (total sugar) and 1.8g/l (pentoses) in hydrolysate in 15-minute autoclave. In 30-minute autoclave sample, hexose and pentose sugar was found to be 9.88g/l and 1.9g/l respectively. Results clearly showed that the doubling the heating period leads to doubling of the sugar released, but the increase mostly was related with hexose and not with pentose sugars **(Figure 3)**. Pretreatment methods such as steam explosion, acid, Alkali, and hot water ones have been extensively applied earlier[20]. Among them, acid pretreatment was found more suitable in many cases including wheat straw[21]. Earlier, 0.7% acid concentration was found suitable for releasing hemicellulosic sugars leaving behind most of the cellulose for further use. An increase of acid concentration leads to digestion of cellulose as well[22] which cannot be good proposition, as cellulose yields pure glucose which can efficiently be fermented to ethanol by yeast while its mixing with hemicellulosic sugars which is mixture of pentoses and hexoses would reduce its fermentation potential.

Lignin removal in the form of phenolics: Phenolics are the degradation products of lignin which on acid treatment get released in acid prehydrolysate called as acid soluble lignin (ASL). The increase in amount of phenolic compound from 0.65mg/g feedstock was found to reach upto level of 0.8mg/g in 30 minute treatment **(Figure 4)**. The increased phenolic compounds release with increase of residence time of heating with pressure indicates the parallelism between hemicellulose sugar release and lignin degradation[12].

Breakdown of oligosaccharides / secondary hydrolysis of oligosaccharides: The amount of total sugars and pentoses increased for the first 10 minutes of heating. After that, the values of sugars remain same or decrease slightly, especially for pentoses; may be due to formation of furans and other derivatives from it.

Detoxification of hydrolysate: Over liming of acidic prehydrolysate not only remove fermentation inhibitors but also change the pH. Over liming is widely used industrial detoxification method[23]. Detoxified hydrolysate by this method show faster fermentation with better cell growth and higher ethanol yield.

Shake flask fermentation: The ethanol yield (4g/l) from treated hydrolysate (14 g/l) medium was found about comparable with unsupplemented medium. There was slight reduction (about 10%) in ethanol yield with 70% treated hydrolysate medium (Table 2), indicating that the residual inhibitors were not diluted enough in this medium to yield ethanol optimally. Detoxification of hemicellulose hydrolysate with lime and Na2SO3 yielded more ethanol than just lime alone. The decrease in ethanol yield with increase in treated hydrolysate concentration from 50 to 70% in the fermentation medium indicated the presence of more inhibitors. Detoxified hemicellulose hydrolysate may be utilised for the production of a range of value-added items such as xylitol, 2,3-butanediol and lactic acid[19].

CONCLUSIONS

The present work clearly demonstrates that though wheat straw may be important source of cellulose for 2G bioethanol, but its hemicelluloses hydrolysate should also be utilized after proper treatment for the production of ethanol for the sake of overall economization of the process.

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