HEMICELLULOSE HYDROLYSATE FROM *AILANTHUS EXCELSA* WOOD POTENTIALLY FERMENTABLE TO ETHANOL

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Hemicellulose hydrolysate from acid- and alkali-impregnated wood chips of *Ailanthus excelsa*, a lignocellulosic substrate, was obtained using heating devices, namely, autoclave and microwave. Effects of various combinations of physical and chemical conditions on pretreatment was assessed based on release of hemicellulose hydrolysate from wood chips and yield of reducing sugars and ethanol from the hemicellulose hydrolysate. The various hydrolysis conditions tested were dilute acid- (0.7% sulphuric acid) or alkali- (3% sodium hydroxide) impregnated wood, two heating devices (autoclave and microwave), either singly or in combination, and single- or two-step hydrolysis process. Single-step acid hydrolysis applying microwave and autoclave as heating method gave almost similar results. The single-step autoclave-mediated dilute acid hydrolysis was chosen as optimal because it yielded the highest amount of pentoses (280 mg g^{-1}) and total sugars (285 mg g^{-1}). Hemicellulose hydrolysate after detoxification was found to be fermentable to ethanol (9.8–10.8 g L^{-1}) by pentose-fermenting yeast, *Scheffersomyces stipitis*. *Ailanthus excelsa* may therefore be a potent tree for bioethanol production.

Keywords: Microwave, autoclave, dilute acid hydrolysis, hydrolysis process

INTRODUCTION

The negative impact of non-renewable fossil fuels on the environment has prompted intensive research in alternative transport fuel. Bioethanol as petroleum extender and octane enhancer in petrol has gained immense importance during the last few decades. Bioethanol has potential not only to reduce the rate of depletion of fossil fuel but also to contribute to environmental amelioration. Bioethanol can be produced from cellulosic biomass which is cheap, renewable and globally available in large quantities (Zhao et al. 2007).

Typical lignocellulosic biomass contains lignin (15–25% w/w), hemicelluloses (23–32%), and cellulose (38–50%) (Mamman et al. 2008). Cellulose is a long-chain homopolymer of glucose, linked together by hydrogen and van der Waals bonds which cause the cellulose to be packed into microfibrils. Hemicelluloses and lignin cover the microfibrils. Thus, although removal of hemicelluloses and lignin is important to expose cellulose for facilitating its subsequent break down into glucose, it is equally important to utilise hemicelluloses efficiently to reduce overall ethanol cost (Hinman et al. 1989). Hemicelluloses are composed of various pentoses and hexoses, of which the major proportion comprises D-xylose (pentose) (Grohmann et al. 1986). Utilisation of hemicelluloses depends largely on the isolation of D-xylose from them for subsequent fermentation.

The first step in bioethanol technology is pretreatment of biomass. Pretreatment removes or alters hemicelluloses and lignin and decreases crystallinity. It also increases porosity or surface area of cellulose to make it more accessible to heat or enzymes that convert carbohydrate polymers into fermentable sugars (Mosier et al. 2005). It has been reported that only less than 20% glucose is released from lignocellulosic biomass without pretreatment while the yield can be as high as 90% with proper pretreatment (Alizadeh et al. 2005). However, pretreatment is often expensive constituting 20% of the overall process cost for lignocellulosic ethanol and releases fermentation inhibitors.

Alkali and dilute acid pretreatments are effective and inexpensive (Sun & Cheng 2005, Zhu et al. 2006). Both of these methods need thermal energy for the hydrolytic process,

which may be supplied by a heating device of contact type (hot plate or heating mantle) or without contact type (microwave), or heat-cum-pressure type (pressure vessel or autoclave) (Nakayama & Okamura 1989, Kitchaiya et al. 2003, Gabhane et al. 2011, Elhaib et al. 2016). A comparison had been made between microwave and other heating sources with respect to their efficacy to hydrolyse garden biomass (Gabhane et al. 2011), wheat straw (Zhu et al. 2006) and corn stover (Shi et al. 2011). The efficacy criteria were the resulting changes in structure of wood, loss of hemicelluloses and lignin and yield of total reducing sugars. Yield of fermentable hemicellulosic sugars and ethanol resulting from pretreatment methods were never considered.

Ailanthus excelsa (Simaroubaceae), also called tree of heaven, is a large deciduous tree, 18–25 m tall and 60–80 cm in diameter. The tree is fast growing, grows well in semi-arid and semi-moist regions and has been found suitable for planting in dry areas with annual rainfall of about 400 mm (Hocking 1993). The bark and leaf are important sources of medicines and wood is used in the manufacture of matches, paper, pulp and plyboard. The wood yields fermentable hemicellulose hydrolysate (Jain et al. 2013). Among the xylose-fermenting yeasts, *Scheffersomyces stipitis* (formerly *Pichia stipitis*) is considered promising for industrial applications due to its ability to ferment xylose with high ethanol yield (Agbogbo et al. 2006). The present work was undertaken to optimise production of hemicellulose hydrolysate from *A. excelsa* wood by impregnating it in acid or alkali followed by application of heat through microwave oven or/and autoclave. The obtained hemicellulose hydrolysate was also examined for its fermentation to ethanol after appropriate treatment using *S. stipitis*.

MATERIALS AND METHODS

Wood

Small branches of *A. excelsa* tree were plucked at the college campus in Bhopal and cut into pieces of about 1 cm long and sheer-milled into small chips (2 mm × 20 mm × 25 mm). The chips were dried at room temperature (30–35 °C) for a week before subjecting them to hydrolysis.

Acid/alkali impregnation

Wood chips (10 g) was put into 250 mL Erlenmeyer flasks followed by 100 mL of 0.7% (v/v) sulphuric acid (H_2SO_4) or 3% sodium hydroxide (NaOH) (v/v) and incubated for 1 hour (Jain et al. 2013). The surplus acid/alkali was decanted before heating.

Single-step cooking with autoclave or microwave

Flasks were autoclaved at 121 °C for 5, 10, 15, 20, 25 or 30 min. In the other experiment, acid-impregnated wood chips were microwaved for the same times at a power of P100.

Two-step cooking with autoclave and/or microwave

Alkali- or acid-impregnated wood chips were cooked using autoclave or/and microwave successively in two steps. After cooking, wood hydrolysate was decanted and collected in separate flasks for sugar analysis. The wood hydrolysate extracted during the two steps was pooled.

Secondary hydrolysis of oligosaccharide

Hydrolysate obtained after various methods of pretreatments (pH 1.3) was separately subjected to heat treatment in boiling water bath at 100 °C for different time intervals. The amounts of total sugars and pentoses released were measured. Distilled water was added to make up any loss of volume.

Detoxification

Acid prehydrolysate (hence after called hydrolysate) with an initial pH of about 1·8 was subjected to detoxification with either lime (overliming) or lime in combination with 0.1% sodium sulphite (Na_2SO_3) until the pH was raised to 11 (Nigam 2002). The mixture was heated for 30 min followed by neutralisation to pH 5.6 by adding 0.1 N hydrocloric acid. Insoluble residues were removed by centrifugation at 10,000 rpm for 10 min, and the supernatant (treated hydrolysate) was collected for further use as fermentable sugars.

Yeast inoculum and inoculation

Scheffersomyces stipitis (NCIM 3507) was used for fermentation of the hydrolysate. The yeast was grown and maintained at 30 and 4 °C respectively on agar slants containing 20 g L^{-1} xylose, 3 g L^{-1} yeast extract, 3 g L^{-1} malt extract, 5 g L^{-1} peptone and 20 g L^{-1} agar. The medium used for inoculum preparation comprised 50 g L^{-1} D-xylose, 5 g L^{-1} glucose, 3 g L^{-1} yeast extract, 3 g L^{-1} malt extract and 5 g L^{-1} peptone and pH was kept at 5. To prepare the inoculum, a 250 mL Erlenmeyer flask containing 50 mL medium was inoculated from a fresh agar slant and incubated at 30 °C in a rotary shaker at 150 rpm for 48 hours prior to use. An amount of 5 mL of this culture was transferred to another 250 mL Erlenmeyer flask containing 100 mL fermentation medium.

Preliminary screening for fermentation of hydrolysate

Preliminary screening for fermentation at different concentrations of treated hydrolysate (v/v), namely, 50, 70 and 100% in fermentation medium containing 10 g L^{-1} yeast extract and 20 g L^{-1} xylose was carried out in large Durham tubes with insert tubes (Wahlbom et al. 2003, Jain et al. 2013). Separate Durham tubes containing unsupplemented treated hydrolysate and xylose-fermentation medium (0.5% w/v yeast extract and 50 mM xylose) were kept as controls. The tubes were filled with 10–15 mL of fermentation medium in triplicate. Each tube was inoculated with 500 µL of fresh yeast suspension of 0.1 optical density taken from an actively growing culture medium. The occurrence of fermentation was marked by liberating bubbles of carbon dioxide gas which were trapped in the inner inverted tube. The tubes were incubated at 25 °C for 2 weeks. Each day they were shaken to help sediment the yeast and examined for gas bubbles.

Fermentation of treated hydrolysate in shake flask

Three different concentrations of treated hydrolysate (in triplicate), viz, 50, 70 and 90% were placed into Erlenmeyer flasks (250 mL) and supplemented with 10 g L^{-1} yeast extract and 20 g L^{-1} xylose. Controls containing unsupplemented hydrolysate medium and xylose fermentation medium were kept concurrently. The media were inoculated as above and kept on a shaker at 150 rpm and temperature 30 °C.

Distillation method

After fermentation the entire 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.

Analytical methods

Assay of pentose (xylose) and reducing sugars was carried out following theorcinol (Plummer 1987) and dinitrosalycilate (Miller 1959) methods. The concentration of lignin degradation product (phenolics) was estimated spectroscopically at 280 nm. Ethanol in the cell-free culture broth and distillate was determined using gas chromatography equipped with flame ionised detector and Total Chrome Navigator software (Kwon et al. 2011). All experiments were conducted in triplicate and results were expressed as means ± standard errors.

RESULTS AND DISCUSSION

Single-step hydrolysis with microwave cooking

Microwave treatment for 30 min yielded maximum amounts of sugars, i.e. 280 mg g^{-1} and 275 mg g^{-1} of total sugars and pentoses respectively (Figure 1a). The amount of phenolics (due to degradation of lignin) also increased correspondingly under this condition (Figure 1b) suggesting that the treatment caused general breakage of hemicelluloses–lignin linkage and subsequent hydrolysis and release of sugars and lignin. Under harsh condition of heating, a small fragment of cellulose also broke down, releasing hexoses.

Single-step hydrolysis with autoclave cooking

The yield of total sugars and pentoses was more in single-step dilute acid treatment after autoclave heating for 30 min, i.e. 285 and 280 mg g^{-1} respectively (Figure 2a). This treatment yielded the highest amount of sugars than the rest of the treatments. Release of phenolics also increased with increase in residence time (Figure 2b). Hardwood of *A. excelsa* free from bark was used

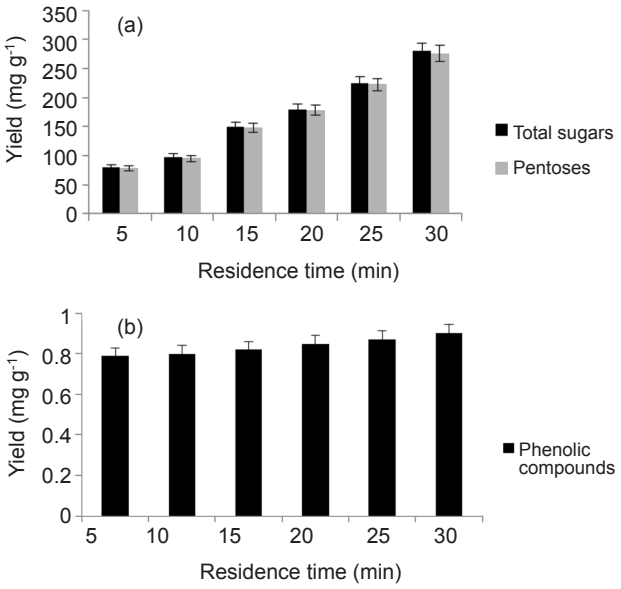

Figure 1 (a) Sugars and (b) phenolic compounds released in the hemicellulose hydrolysate using microwave as heating method; values are means ± standard errors from experiments in triplicate

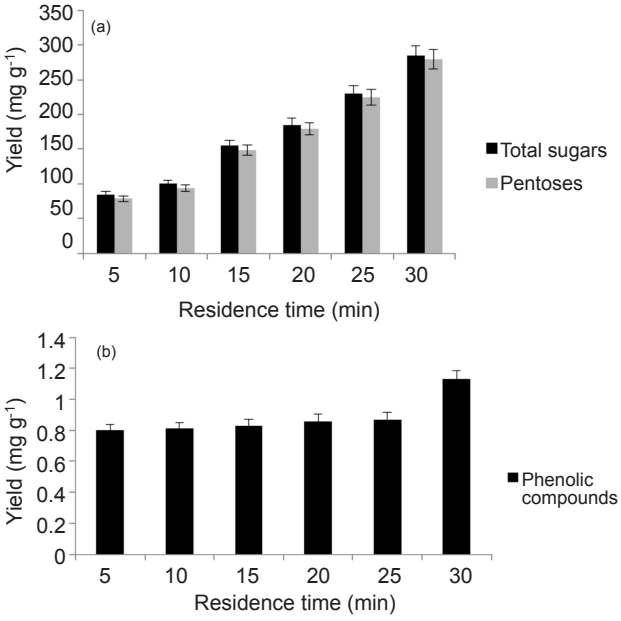

Figure 2 (a) Sugars and (b) phenolic compounds released in the hemicellulose hydrolysate using autoclave as heating method; values are means ± standard errors from experiments in triplicate

in the experiment. The acid concentration (0.7% H_2SO_4) used in this study was reported to be optimum for this analysis (Jain et al. 2013). Acid is one of the most important factors in releasing sugars from wood. Sugars were charred when higher concentrations of reagents (10% H_2SO_4 or NaOH) were used (Table 1, Jain et al. 2013). Higher concentrations of acid may decompose the hemicellulosic structure, producing inhibitors and also cause damage to the equipment used. In addition, the consumption of lime to raise the pH will also increase. Therefore, an appropriate acid concentration is essential for acid hydrolysis of lignocellulose at industrial scale (Taherzadeh & Karimi 2007).

The efficiency of heating instruments (autoclave and microwave) was checked individually and in combination for extraction of hemicellulosic sugars. The single-step autoclave-mediated dilute acid hydrolysis was found to yield the highest amount of pentoses (280 mg g^{-1}) and total sugars (285 mg g^{-1}) (Figure 2). When under pressure, heat makes the substrate fragile for further breakdown, hence releasing more sugars subsequently (Torget & Hsu 1994). The increase in the yield of sugars with increase in residence time up to 30 min is in accordance with findings by Gabhane et al. (2011). The increase in the amount of phenolic compounds (degradation products of lignin) and sugars with the increase of residence time indicates a parallelism between the release of hemicelluloses and lignin (Gabhane et al. 2011). Both components were intimately associated with each other in the wood and thus both seemed to be released together in response to pretreatment. Therefore, the presence of lignin and its degradation products in the hemicellulose hydrolysate was not avoidable at the pretreatment level.

Using microwave was as effective as autoclave, although the yield of sugars was a little less. This suggests that pressure is important to release sugars (Gabhane et al. 2011). Microwave-oven mediated dilute acid pretreatment yielded maximum amount of sugars in 30 min (Table 1). Microwave irradiation destroys the crystallinity of cellulose and degrades lignin and hemicelluloses (Kitchaiya et al. 2003). It enhances the accessibility of the materials for enzymatic as well as for acid and alkali hydrolyses (Chen et al. 2011). However, at the same time it has been rated as less superior to a pressure vessel in hydrolysing wood and in releasing phenolic by-products (Torget & Hsu 1994). Higher pressure during treatment caused degradation of some cellulose, resulting in more released sugars and less left over cellulose. Lignin degradation was found to depend upon severity

Table 1 Sugars in the hydrolysate obtained by two-step hydrolysis of alkali- or acid-impregnated wood chips using microwave or autoclave

Hydrolytic condition	Heating device	Pentoses (mg g^{-1})	Total sugars (mg g^{-1})
NaOH (3%)	Autoclave	63.3 ± 0.75	84.2 ± 0.05
H_2SO_4 (10%)	Autoclave	Charred	Charred
H_2SO_4 (10%)	Microwave	Charred	Charred
H_2SO_4 (1%)	Microwave	156.2 ± 0.45	163.3 ± 0.81
H_2SO_4 (0.6%)	Autoclave	181.0 ± 0.31	193.0 ± 0.08
H_2SO_4 (0.7%)	Autoclave	284.5 ± 0.01	295.3 ± 0.15
H_2SO_4 (0.8%)	Autoclave	212.9 ± 0.01	215.9 ± 0.08
H_2SO_4 (0.9%)	Autoclave	192.8 ± 0.01	213.6 ± 0.01
H_2SO_4 (1%)	Autoclave	176.0 ± 0.81	204.0 ± 0.16

Total residence time 30 + 30 min; values are means ± standard errors from experiments in triplicate

of treatment, i.e. increase in acid concentration and residence time in autoclave.

Dilute acid/alkali hydrolysis with combined microwave and autoclave cooking

Maximum amounts of pentoses and total sugars obtained from dilute acid-impregnated wood using combined treatment of microwave oven and autoclave were 20.2 mg g^{-1} and 23.3 mg g^{-1} respectively. Cooking with 3% NaOH-impregnated wood generated less sugars while 10% NaOH-impregnated wood resulted in charring of sugars (Table 1). Alkali pretreatment with NaOH yielded very less sugars compared with dilute acid pretreatment (Table 1) because alkali extracts released mostly phenolic compounds and very less hemicellulosic sugars (Kumar et al. 2009). On the other hand microwave or autoclave treatment of alkali-impregnated wood produced almost similar amounts of sugars and phenolic compounds. This confirmed the parallel effect of microwave and autoclave on the release of wood components.

Alkali pretreatment is suitable for biomass with very high lignin content (Mosier et al. 2005) such as agrowastes and where there is no emphasis on recovery of hemicellulosic sugars. Chemical conditions under which the biomass was treated were important for good yield of sugars but heating methods (microwave or autoclave) did not produce any difference in results.

Secondary hydrolysis of oligosaccharide

The amounts of total sugars and pentoses increased for the first 15 min of heating. After that, the values were either the same or decreased slightly, especially for pentoses.

Detoxification of hydrolysate

Lime in combination with Na_2SO_3 was effective in removing fermentation inhibitors from hydrolysate. Detoxified hydrolysate by this method exhibited faster fermentation.

Shake flask fermentation

The ethanol yield from 50% treated hydrolysate medium was 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 Na_2SO_3 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 (Saha 2003).

CONCLUSIONS

The present work focused on the extraction of hemicellulosic sugars from a lignocellulosic source, *A. excelsa*, for conversion to ethanol. Dilute acid-impregnated wood of *A. excelsa* heated with either microwave or autoclave yielded

Table 2 Utilisation of sugar and ethanol production from fermentation medium containing treated hydrolysate (50 or 70 % v/v) detoxified by two different methods by *Scheffersomyces stipitis*

Treated hydrolysate (%)	Treatment method	Total sugars (g L^{-1})		Pentoses (g L^{-1})		Ethanol (g L^{-1})
		Initial	Residual	Initial	Residual	
50	Lime	30.9	2.8	22.2	3.1	4.2
50	Sodium sulphite + lime	32.9	2.1	24.8	2.9	10.8
70	Lime	34.5	4.4	26.8	4.0	2.2
70	Sodium sulphite + lime	35.8	2.4	28.6	3.1	9.8

almost equal amounts and types of sugars. As the amount of sugars increased, the amount of products from lignin degradation, i.e. phenolics also increased. Detoxified hydrolysate could be fermented to ethanol using pentose-fermenting yeast *S. stipitis*. *Ailanthus excelsa*, a fast-growing and draught-tolerant species, has great potential as a valuable energy tree.

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