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PRODUCTION OF FERMENTABLE SUGARS BY COMBINED CHEMO-ENZYMATIC HYDROLYSIS OF CELLULOSIC MATERIAL FOR BIOETHANOL PRODUCTION

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Abstract - To change the recalcitrant nature of the lignocellulosic material for maximum hydrolysis yield, a comprehensive study was done by using sulphuric acid as an exclusive catalyst for the pretreatment process. The enzymatic digestibility of the biomass [Water Hyacinth: *Eichhornia crassipes*] after pretreatment was determined by measuring the hydrolysis yield of the pretreated material obtained from twenty four different pretreatment conditions. These included different concentrations of sulphuric acid (0.0, 1.0, 2.0 and 3.0%), at two different temperatures (108 and 121 °C) for different residence times (1.0, 2.0 and 3.0h).The highest reducing sugar yield (36.65 g/L) from enzymatic hydrolysis was obtained when plant material was pretreated at 121 °C for 1.0 h residence time using 3.0% (v/v) sulphuric acid and at 1:10 (w/v) solid to liquid ratio. The total reducing sugars obtained from the two-stage process (pretreatment + enzymatic hydrolysis) was 69.6g/L. The resulting sugars were fermented into ethanol by using *Saccharomyces cerevisiae*. The ethanol yield from the enzymatic hydrolyzate was 95.2% of the theoretical yield (0.51g/g glucose), as determined by GS-MS, and nearly 100% since no reducing sugars were detected in the fermenting media by TLC and DNS analysis.
Keywords: *Eichhornia crasspies*; Lignocellulosic; Water hyacinth; *Saccharomyces cerevisiae*; Ethanol; Fermentation.

INTRODUCTION

Lignocellulosic biomass is an abundant, inexpensive and readily available source of fermentable sugars (Ho *et al*., 1998). For the last few decades, the conversion of these resources into glucose and other reducing sugars has been considered as an attractive route for production of ethanol or other valuable chemicals (Curreli *et al*., 1997; Gaspar *et al*., 2005). A wide array of biomass sources, including agricultural residues such as corn stover, wheat and rice straw and forestry residue; industrial residues such as pulp and paper processing waste and energy crops such as switchgrass have been employed as biomass source. However, unlike starch, which contains homogenous and easily hydrolyzed biopolymers, lignocellulosic plant matter contains cellulose (23-53%), hemicellulose (20-35%), and polyphenolic lignin (10-25%). Glucose, obtained from lignocellulosic material, is usually expected to be a renewable source, which

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can be efficiently converted into fuels, foods, and other valuable chemicals (Huber *et al*., 2006; Klemm *et al*., 2005; Fan *et al*., 1987; Zhang and Lynd, 2004; Ragauskas *et al*., 2006; Davda and Dumesic, 2005). Cellulosic conversion into glucose therefore is a key process which needs to be further studied.

The selection of the biomass source is of great importance from a technical and economical point of view. Ethically, biomass should not compete with the food crops, and thus waste biomass or crops with low commercial value, such as agricultural waste or weeds, are preferred for such types of processes for producing valuable chemicals. Furthermore, it is important to select a source that requires less fertilizer, has a high growth rate and is preferably available the whole year, as is the case with water hyacinth. Water hyacinth could be an excellent biomass feedstock for further conversions and utilization (Girisuta *et al*., 2008).

Water hyacinth is a fast growing aquatic weed present in water reservoirs such as large lakes, rivers, shallow ponds, wetlands and marshes (Naseema *et al*., 2004). It can double its mass within 8-10 days and a single plant can produce up to 3000 offspring in 50 days (Verma *et al*., 2003). Because of its capacity for exponential increase in the biomass, this weed needs constant vigilance by farmers and canal irrigation personnel. Because of its enormous amount, some uses have been suggested such as composting, cattle feed, biogas plant resources, paper and pulp industry, furniture making, and waste water treatments (Gunnersson and Petersen, 2007). However, there is no reported utilization of this weed on a large industrial scale and it still continues to be jeopardy for farmers and water management authorities as it blocks water flow in irrigation and drainage canals, channels and streams. This weed also makes aquatic recreational activity difficult and is potentially unsafe in lakes, thus causing potential hurdles to tourism and related industries. In an attempt to address such problems, we propose to use it as a raw material for the extraction of fermentable sugars for value-added chemicals by optimizing and studying the two-stage hydrolysis process using commercial cellulase enzyme.

MATERIALS AND METHODS

Chemicals

3,5-Dinitrosalicylic acid and analytical grade phenol were received from Fluka Chemie, D(+)-xylose(GPR) was obtained from BDH (England) and L-(+)-arabinose from Sigma Aldrich. Concentrated sulphuric acid (95–97 wt.%) and D(+)-glucose were obtained from Panreac and α-naphthol was purchased from Merck (Darmstadt, Germany). Distilled water was used to prepare the various solutions. Accellerase^{TM}1500 (Cellulase) having multiple enzyme activities; exoglucanase, endoglucanase, hemicellulase and beta-glucosidase as reported by the manufacturer, was obtained from Genencore International (U.S.A).

Water Hyacinth

Fresh and healthy water hyacinth plants were collected from a natural pond near Shahdrah, Lahore (Punjab, Pakistan), during December. They were thoroughly washed with tap water several times to remove adhering dirt. Samples of stem, petiole and leaf of the fresh plant were selected as the substrate for saccharification. These parts were dried in an oven at 105 °C for 20 min and subsequently chopped into small pieces (~1-2 cm) and blended to small particles (~3-5 mm).

Pretreatment of the Water Hyacinth

The powdered dry plant material was used for pretreatment under different conditions. Twenty five grams of water hyacinth powder were mixed separately with 0.0, 1.0, 2.0 and 3.0% H_2SO_4 solution in 1:10 w/v% ratio, in 500 mL flasks. The flasks were autoclaved (CL-40L (ALP Co, Ltd. Tokyo, Japan) at two different temperatures (108 °C and 121 °C at a pressure of 0.11 MPa) for different time intervals (1.0, 2.0 and 3.0h). The solutions in the flasks were cooled and filtered with Whatman filter paper. The residue was washed with distilled water 3-5 times to bring the pH at 4.8. The residue was dried at 105 °C for 20 min and weighed.

Enzymatic Hydrolysis of Pretreated Material

The enzymatic hydrolysis was carried out in 250 mL glass flasks using solid biomass residue obtained after each pretreatment condition. A specific volume of enzyme (0.2 mL/g dry weight of biomass) was used for hydrolysis. Five grams of pretreated dry powder of water hyacinth were added in each flask separately. The pH of the reaction mixture was set at 4.8 by adding 100mL of 0.1M acetate buffer solution. The flasks were kept in an orbital shaker for 48.0 hours at 50 °C and 160 rpm. At regular time intervals, sample were taken from each flask and kept in boiling water to inactivate the enzyme. Each

sample was filtered on Whatman filter paper and subsequently analyzed. Each experiment was performed in duplicate.

Ethanol Fermentation

Commercial Baker's yeast (*Saccharomyces cerevisiae*) obtained from a local market, was used for the ethanol fermentation. Inoculum was prepared by transferring yeast cells (1.0g/100mL) into 250 mL flasks containing 50.0 mL of culture medium containing 10.0 g/L yeast extract, 20.0 g/L peptone, and 20.0 g/L glucose and subsequently incubating at 30.0 °C for 24.0 h. This was used to inoculate the fermentation medium. Cellulosic hydrolyzate, obtained from enzymatic hydrolysis, was supplemented with 1.0 g/L yeast extract, 2.0 g/L $(NH_4)_2SO_4$ and 1.0 g/L of $MgSO_4$. The inoculum-to-solution ratio of 1:10 was used for fermentation purposes. Samples for glucose and ethanol analysis were taken at the beginning and end of a 24.0 h fermentation process.

Analysis of Reducing Sugars and Ethanol

The amount of the reducing sugars was determined using 3,5-dinitrosalicylic acid (DNS) reagent by the Ghose method (1987). Identification of monomeric sugars was done with thin layer chromatography (TLC) by using alpha-naphthol as locating reagent and a water: acetonitrile mixture (85:15) as eluting solvent, as used by Beom *et al*. (2009) and outlined in Idrees *et al*. (2013). The hydrolysis yield was calculated on the basis of pretreated solid biomass used for enzymatic hydrolysis. Statistical analysis was done with Graph Pad Prism 5.

After centrifuging the liquid from the fermentation media for 10 min, ethanol was quantified in the supernatant with the help of GC-MS (GCMS-QP2010 of Shimadzu) using a DB-5 capillary column (diameter 0.25 mm, length 30.0 m and thickness 0.25 μm). Nitrogen was used as carrier gas with flow rate of 1.41 mL/min. The temperature program was: temperature maintained at 40 °C for 1.0 min, then raised to 44.0 °C at 15.0 °C/min and at 1.0 °C/min up to 50.0 °C, then continuously increased to 250.0 °C at 25.0 °C/min and finally held at 250.0 °C for 2.0 min. The ion source temperature was 200.0 °C. Data was obtained in the scan mode in the mass range of 30-120 m/z after injecting 2.0 μL of sample. Fragment ions 31 m/z and 45 m/z were used for identification and quantification of ethanol, respectively. The calibration curve was obtained from 0.1, 0.2, 0.3 up to 1.0% v/v concentration of ethanol in HPLC grade water and their peak areas. From this, the concentration of ethanol (v/v) in the sample was determined, which was converted to (w/v) by multiplying it by 0.79 (specific gravity of ethanol at 20.0 °C).

RESULTS AND DISCUSSION

Water hyacinth has a high cellulosic content (40.0-65.0%) (Malik, 2007; Nigam, 2002) with extremely high growth rate (140 ton/ha. year, dry wt.) and have been considered as a prospective source for production of ethanol and other fuels (Girusta *et al*., 2008; Abraham *et al*., 1996; Sherma *et al*.,1999; Singhal and Rai, 2003). To obtain the maximum enzymatic hydrolysis for the production of ethanol, a pretreatment step which consumes cheap chemicals is necessary for process economy. The pretreatment has the capability to decrease the crystallinity of the cellulose and hemicellulose, remove the lignin content and avoid the production of potential inhibitors for fermenting organisms. Dilute acid pretreatment using sulphuric acid below 4.0% was considered to be an economical method, providing higher hydrolysis yield among the different physicochemical pretreatments (Esteghlalian *et al*., 1997). The process has been conducted in the temperature range of 100 °C - 200 °C with pressure 15Psi to 75Psi for different time intervals (Gangulya *et al*., 2012). We have used sulphuric acid for pretreatment to enhance the hydrolysis yield at different temperatures and pressures for varying times. Accellerase^{TM} 1500 was used at specific concentrations to investigate the enzymatic hydrolysis performance of cellulose and hemicellulose present in the treated water hyacinth plant. The conversion of lignocellulosic material into reducing sugars and liquid fuel (ethanol) has been achieved by using three sequential steps: acid pretreatment, enzymatic hydrolysis and yeast fermentation.

Effect of Biomass Concentration on Hydrolysis Yield

The effect of biomass concentration was investigated on the enzymatic hydrolysis yield and amount of reducing sugars by using different quantities of pretreated biomass with fixed concentration of the enzyme (0.5 mL enzyme). With the increase in substrate concentration, the amount of the reducing sugars increases while the hydrolysis yield decreases, showing an opposite variation trend (Fig. 1). At low concentration (5.0 g/L) of substrate, the hydrolysis yield is maximum corresponding to 96.0%, which decreases to 39.0% when the quantity of biomass increased to 125.0 g/L (Fig. 1). The low hydrolysis

yield at high substrate loading was due to two reasons, the lower enzyme to substrate ratio and inhibition of end product feedback caused by the high concentration of reducing sugar produced during hydrolysis (Wen *et al*., 2004). The effect of the substrate on the amount of the reducing sugars and hydrolysis yield was significant (p value<0.0001) with R^2 value 0.9909. The optimum biomass concentration was determined from the amount of the reducing sugars and the hydrolysis yield. At 40.0 g/L solid mass the hydrolysis yield is 74.0%, which decreases at 70.8% when the substrate amount increases to 50.0 g/L and 58.0% with 75.0 g/L substrate. So fifty grams per liter substrate is effective for enzymatic hydrolysis.

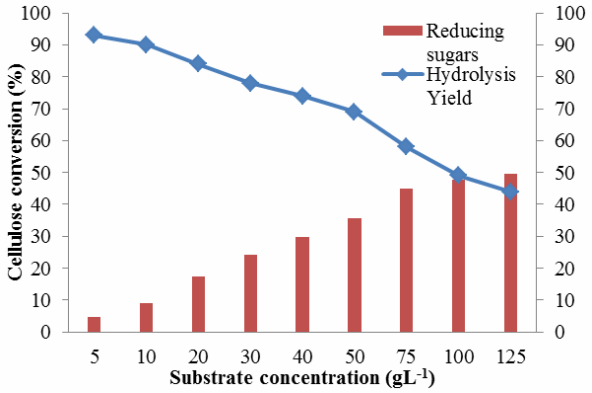

Figure 1: Effect of substrate concentration on the enzymatic hydrolysis at fixed ratio of enzyme.

Effect of Time and Temperature on Pretreatment

The effect of the time and temperature on the pretreatment and enzymatic hydrolysis was prominent. The amount of cellulosic residue left after each pretreatment was different for varying pretreatment conditions (Table 1). At high temperature and longer residence time, the maximum hydrolysis of the hemicellulose was observed in the pretreatment steps, which resulted in decreasing the remaining polysaccharides in the biomass. After pretreatment with 3.0% acid at low temperature (108 °C), the solid residue left was higher (50.32%) which have more hemicellulose content while at high temperature (121 °C) the residue was less (35.45%), due to maximum hydrolysis of hemicellulose into component sugars. During sulphuric acid pretreatment glucose, arabinose and xylose were obtained from the hydrolyzate, which contains glucose as major component as shown by TLC. Previously it was claimed that the acid hydrolyzate of water hyacinth plant contains xylose as major component (Nigam, 2002). At 121 °C and 3.0 h of pretreatment, the reducing sugars produced from hemicellulose (Ackerson *et al*., 1981; Taherzadeh and karimi, 2007) were converted into furfural (Fig. 2B). The production of furfural depends on the acid concentration and temperature (Gonzales, 1986). During enzymatic hydrolysis, the quantity of reducing sugars increases with increase in time of pretreatment for 1.0% and 2.0% acid and decreases with 3.0% acid due to the lower quantity of hemicellulose present in the pretreated biomass. The maximum amount of reducing sugars (hydrolysis yield) was obtained from water hyacinth when it was pretreated with 1.0% acid for 3.0 h and 3.0% acid for 1.0 h (Fig. 3(a), (b)).

Effect of Acid Concentration on Hydrolysis

Cellulose related polysaccharides are considered to be a major component of water hyacinth (Malik, 2007; Nigam, 2002; Mukherjee and Nandi 2004; Ingole and Bhole, 2002). The plant body contains 26.3 wt% C-6 sugars such as glucose (19.8%) and galactose (6.5%) and 20.5 wt% C-5 sugars with 11.5 wt% xylose and arabinose (Girisuta *et al*., 2008; Aswathy *et al*., 2010). The pretreatment conditions had a significant influence on the amount of sugars released during pretreatment step and enzymatic hydrolysis. In acid pretreatment the cellulose and hemicellulose hydrolyzed into reducing sugars. The hydrolysis of hemicellulose increases with the increase in concentration of acid used for pretreatment, which also results in more of a decrease in residual mass (Table 1). The reduction in mass of water hyacinth increased from 2.62 % to 64.55% when the acid concentration was increased from 0.0 to 3.0% during pretreatment. This reduction in weight during pretreatment was due to removal of metal oxides (Girisuta *et al.,* 2008), lignin and hydrolysis of hemicellulose. Pretreatment of water hyacinth with sulphuric acid yields a mixture of sugars (glucose, xylose, arabinose), with glucose as the major component (Fig. 2A and 2C), which exactly corresponds to the results of acid hydrolysis of water hyacinth leaves by Girisuta *et al.* (2008). The amounts of the reducing sugars obtained after enzymatic hydrolysis, when pretreatment was done with 0.0, 1.0, 2.0 and 3.0% sulphuric acid at 121 °C for 1.0h, were 1.15±0.12, 30.38±0.77, 31.85±0.8 and 36.68±0.82 g/L respectively. Similarly, 1.77±0.07, 35.29±1.4, 32.03±1.09 and 26.26±1.19 g/L of reducing sugars were obtained from water hyacinth biomass when pretreatment was done with 0.0, 1.0, 2.0 and 3.0% sulphuric acid at 121 °C for 3.0 h (Table 1). The sugars obtained during enzymatic hydrolysis were almost pure glucose (Fig. 5A and 5B).

The effect of the acid concentration on the amount of the reducing sugars was significant (P<0.0001) with R^2 value 0.9709 calculated from ANOVA analysis using Graph PadPrism5. It was clear that the hydrolysis yield increases with the increase in acid concentration with short pretreatment time and decreases with long pretreatment time. The 3.0% acid pretreatment gave a higher amount of reducing sugars when pretreated for 1.0 h and less when treated for 3.0 h. This was due to the complete hydrolysis of the hemicellulose and charring of the remaining cellulose in the pretreatment step.

Table 1: The reaction conditions, biomass residue and amount of fermentable sugars obtained after enzymatic hydrolysis.

Acid Conc. (%)	Pretreatment Time (h)	Temperature (°C)	Decrease in Biomass (%)	Biomass Residue (%)	Reducing Sugars g/L (Pretreatment step)	Reducing Sugars g/L (Enzymatic Step)	Enzymatic Hydrolysis Yield (%)
0.0	1	108	2.64	97.36±1.12	0.57±0.23	1.04±0.14	2.1
	1	121	16.18	83.82±1.71	2.42±0.58	1.150±.12	2.31
	2	108	5.04	94.96±3.78	0.81±0.34	0.95±0.08	1.9
	2	121	26.44	73.56±0.88	2.99±2.01	1.62±0.19	3.24
	3	108	25.32	74.68±2.46	3.15±1.32	1.45±0.21	2.9
	3	121	35.85	64.15±1.97	5.21±1.28	1.76±0.07	3.55
1.0	1	108	47.19	52.81±1.17	23.41±1.37	28.80±0.93	57.61
	1	121	57.03	42.97±0.94	31.29±0.96	30.36±0.77	60.77
	2	108	50.26	49.74±1.11	21.72±0.89	29.97±1.61	59.8
	2	121	59.05	40.95±0.13	29.03±2.14	32.38±1.01	64.72
	3	108	51.43	48.57±0.93	25.47±1.22	31.60±0.80	63.48
	3	121	61.37	38.63±0.24	27.25±2.40	35.30±1.40	70.56
2.0	1	108	48.84	51.16±1.96	21.83±1.34	29.12±1.13	58.24
	1	121	61.33	38.67±0.64	32.76±0.96	31.85±0.85	63.58
	2	108	49.12	50.88±0.66	20.12±2.11	30.75±1.40	61.6
	2	121	63.33	36.67±1.16	31.31±1.58	29.10±0.90	57.8
	3	108	50.15	49.85±0.70	24.38±1.76	29.59±1.19	59.56
	3	121	61.54	38.46±0.86	29.71±2.80	32.03±1.09	63.98
3.0	1	108	47.94	52.06±0.45	28.58±1.36	30.75±1.83	61.51
	1	121	61.97	38.03±1.96	33.47±2.10	36.68±0.82	73.4
	2	108	48.98	51.02±1.26	29.19±1.59	31.30±1.06	62.6
	2	121	63.26	36.74±0.24	32.76±1.16	31.67±0.94	63.34
	3	108	49.68	50.32±0.84	26.93±2.11	29.25±0.83	58.6
	3	121	64.55	35.45±1.11	29.43±1.73	26.26±1.19	52.7

Experiments were done in duplicate. The average values and standard deviation are shown.

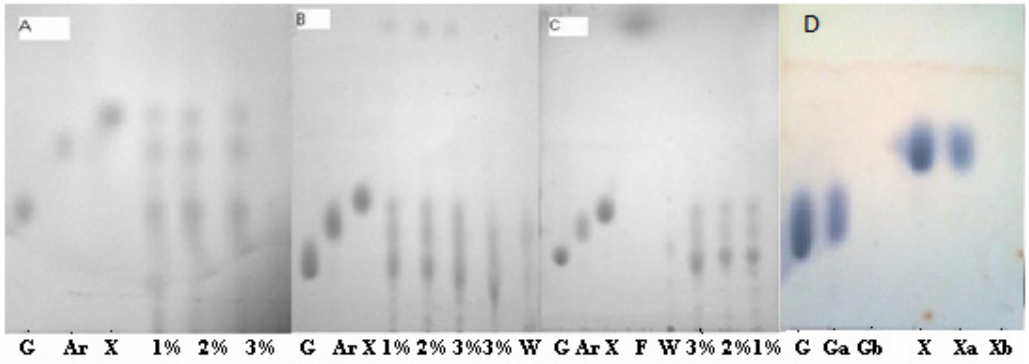

Figure 2: Reducing sugars in the acid hydrolyzate obtained during pretreatment (TLC images) A: 108 °C for 3.0h, B: 121 °C for 3.0h and C:121 °C for 1.0h,W: water, G: glucose, X: xylose, Ar: arabinose, F:furfural, D: Effect of sulphuric acid and NaOH on glucose and xylose(a:Acid, b: NaOH).

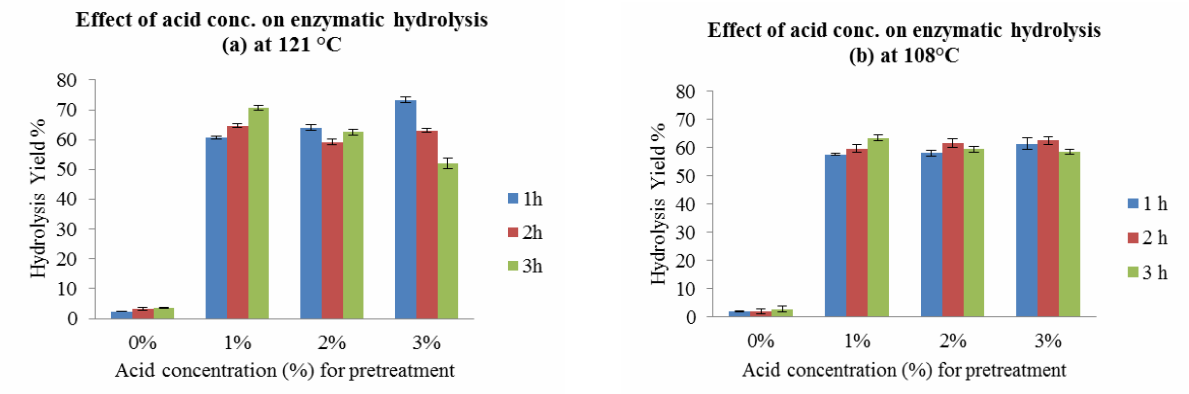

Figure 3: (a), (b). Effect of acid concentration and time of pretreatment on enzymatic hydrolysis (%).

Time Course of Enzymatic Hydrolysis

Enzymatic hydrolysis was done with a fixed amount of the cellulase enzyme and the reaction carried out for 48.0 h. The reducing sugar concentrations were determined at regular intervals, (12.0 h) starting from 0 h during hydrolysis. The amount of the sugars increased gradually and reached 0.734 g/g of cellulosic material, which corresponds to a 73.4% hydrolysis yield after 48.0 h, as shown in Fig. 4. The percentage of hydrolysis was calculated from the amount of reducing sugars and the amount of pretreated biomass used in enzymatic hydrolysis. The graph showed that, with the passage of time, the amount of reducing sugars increased and after 48.0 h there was an insignificant increase in the amount of sugars observed in some experiments. During hydrolysis, in the first 24.0 h more sugars were obtained and then sluggishly increased and reached a maximum at 48.0 h. Previously 71.3% enzymatic saccharification efficiency was reported by Aswathy *et al*., (2010) with NaOH pretreated water hyacinth and 60.2% by Mishima *et al*., (2008).

Most Effective Pretreatment Condition

Acids such as sulphuric acid, hydrochloric acid or nitric acid (Patel *et al*., 1993) and bases like NaOH or NH_3 (Zhao *et al*., 2007; Xu, 2007) can be used efficiently for pretreatment of biomass at different temperatures for maximum enzymatic hydrolysis. Among them, NaOH pretreatment could provide higher enzymatic saccharification as compared to acids (Zhao *et al*., 2007; Aswathy *et al*., 2010). During acidic or basic pretreatment, there occurred a loss in the biomass weight (Jiele *et al*., 2010; Wang *et al*., 2009) due to hydrolysis of hemicellulose and removal of lignin (Blasi *et al*., 1999; Lin *et al*., 2010).

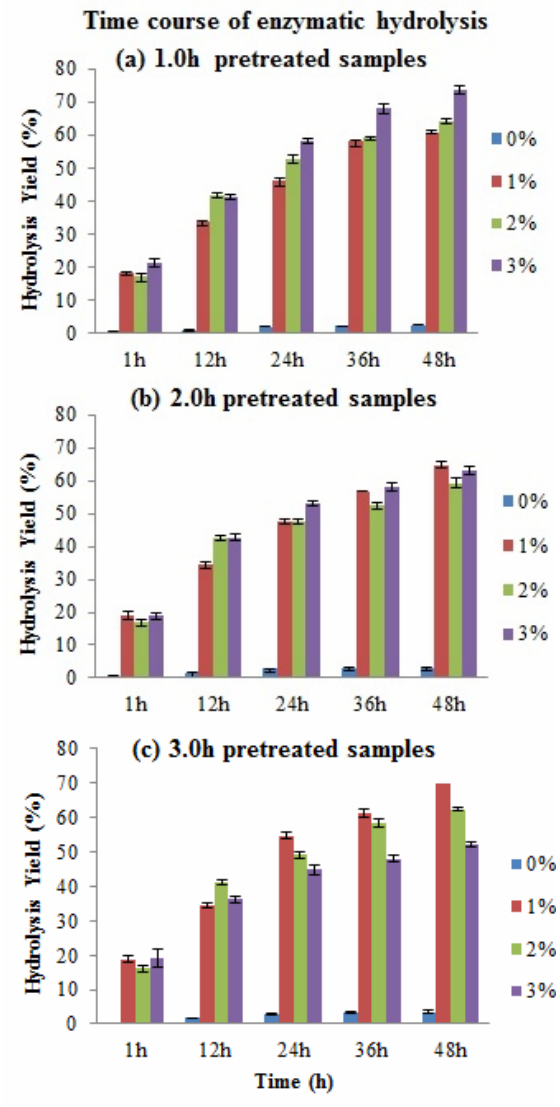

Figure 4: Time course of enzymatic hydrolysis when biomass was pretreated at 121 °C with different concentrations of sulphuric acid (0%, 1%, 2%, and 3%).

Water hyacinth contains hemicellulose as major component (48.0%) with only 3.5-4.6% of lignin (Nigam, 2002), which was removed during the pretreatment step (decrease in biomass weight Table 1). For obtaining the maximum benefit of pretreatment the sugars produced during pretreatment should be stable. The effect of both H_2SO_4 and NaOH on pure glucose and xylose at 100 °C was checked. NaOH degraded these sugars, while in acidic media no change was observed for glucose and xylose (Fig. 2D). So the use of H_2SO_4 at low temperature for pretreatment was a better choice for obtaining the maximum amount of fermentable sugars in two steps, pretreatment and enzymatic hydrolysis step. The pretreatment of water hyacinth with 3.0% sulphuric acid at 121 °C for 1.0 h was found to be the most optimal condition as the subsequent enzyme hydrolysis showed maximum 73.4% yield. This pretreatment also provided 33±2.1 g/L of reducing sugars per 100 gm of biomass during pretreatment step, which is close to the results obtained by Abraham *et al.* (2006) by using 10.0% sulphuric acid at 121 °C for 30 min, which was also available for fermentation (Masami *et al.*, 2008). Nigam (2002) obtained 0.51 g/g reducing sugars with 3.0% sulphuric acid when the pretreatment time was 1.5 h. The pretreatment with 1.0 and 2.0% sulphuric acid for 3.0 h at 121 °C provided 27.25± 2.4 and 29.19±2.8 g/L of reducing sugars per 100.0 g of biomass, along with 70.56 and 63.98**%** enzymatic hydrolysis yields, respectively.

Ethanol Production

Two types of enzymatic hydrolyzate, one obtained from 1.0% acid and other from 3.0% acid treated biomass, were used for ethanol production. These two hydrolyzates have 36.65 g/L and 35.7 g/L fermentable sugars which converted into 18.25 and 17.33 g/L ethanol, equivalent to 95.2% of the theoretical yield of the glucose, which is 0.51 g ethanol/ g of glucose. After twenty four hours, sugars were entirely fermented into ethanol, which was confirmed through DNS analysis and TLC results (Fig. 5C). Sornvoraveat and Kongkiattikajorn (2010) obtained a 96.07% ethanol yield from fermentation of enzymatic hydrolyzate of water hyacinth and Chen *et al*. (2007) obtained a 94.0% yield from corncob enzymatic hydrolyzate in 18.0 h, by using *Saccharomyces cerevisiae*. Nigam (2002) and Magdum *et al*., (2012) reported 18.0 g/L and 19.2g/L of ethanol from the acid hydrolyzate of water hyacinth leaves, respectively. Sulphuric acid produced pure cellulose after hydrolysis of the hemicellulose during pretreatment. Enzymatic saccharification of this cellulose produced pure glucose (Fig. 5) which converted completely into ethanol through fermentation by using commercial yeast.

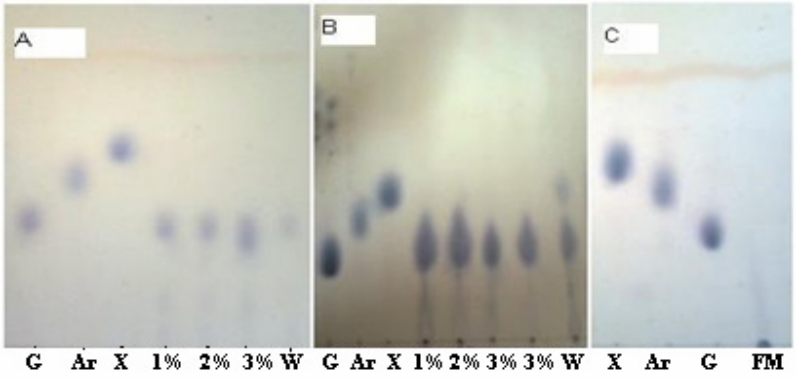

Figure 5: Monomeric sugars after enzymatic hydrolysis: "A" Enzymatic hydrolysis after pretreatment at 108 °C for 1h, "B" enzymatic hydrolysis after pretreatment at 121 °C for 1.0 h and "C" after fermentation (W: water, G: glucose, X: xylose, Ar: arabinose, FM: fermentation media).

CONCLUSIONS

Chemo-enzymatic hydrolysis of the water hyacinth yielded reducing sugars in two steps: (a) Pretreatment step yielded 33.0% hydrolysis; (b) Enzymatic hydrolysis yielded 73.4%. Multiple enzyme activities of Accellerase™1500 converted the cellulose and cellubiose completely into pure glucose. The amounts of glucose obtained from enzymatic hydrolysis of pretreated water hyacinth with 3.0% and 1.0% acid were 36.65 and 35.7 g/L, which gave 18.25 and 17.33 g/L of ethanol with commercial baker's yeast respectively. The two-step hydrolysis process of water hyacinth, pretreatment with sulphuric acid followed by Accellrase 1500 hydrolysis, is a suitable method for achieving high recovery of fermentable sugars and high ethanol conversion yield.

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