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OPTIMIZATION OF ACID AND ENZYMATIC SACCHARIFICATION OF LIGNOCELLULOSIC SUBSTRATE WATER HYACINTH (*EICHHORNIA CRASSIPES*)

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Abstract:-Bioethanol production from lignocelluloses is one of the emerging topics worldwide due to limited sources of fossil fuel and associated environmental problems like global warming and climate change. Water hyacinth (*Eichhornia crassipes*) with 10% lignin and 65% holocellulose content , is a fast growing aquatic plant exists all over the world. It is a low cost, renewable and a potential source of lignocellulosic feed stock for bioethanol production. In the present work, acid saccharification carried out at optimum condition of temperature (120°C), acid concentration (2% v/v), biomass loading (1:10 w/v) and time (60 min) yielded 280±11.2 mg/g of tota sugars. Further, enzymatic saccharification of remaining substrate under optimum conditions of temperature 50°C, 150 rpm, biomass loading (1: 5), surfactant concentration (Triton) 0.1% and enzyme accelerase 1500 yielded 340±13.8 mg/g of total sugars. The combined hydrolysis (acid and enzyme) of water hyacinth under optimized conditions resulted in 95.4% of hydrolysis.

Keywords: Lignocellulose, *Eichhornia crassipes*, Acid hydrolysis, Accelerase 1500, Enzyme hydrolysis

INTRODUCTION

Among green house effecting gasses, emission of CH_4, NO_2, CO globally constitute approximately 98% of the total emissions and their share is expected to increase significantly in this twenty-first century. Carbon dioxide (CO_2) represents 77% of the total global greenhouse gas emissions (Juan C et al 2013), while methane (CH) and nitrous oxide (N_2O) are emitted to a lesser extent i.e. 14 and 8% but exhibit global warming potentials 23 and 298 times higher than that of carbon dioxide respectively (Intergovern mental Panel on Climate Change 2007). To reduce global warming most countries of the United Nations have committed themselves to significantly reduce their greenhouse gas (GHG) emissions without any effect on industrial growth. If properly tailored a low-cost and environmental friendly method, alternative to physical/ chemical ways for the reduction of GHGs can be carried out by means of biotechnological routes (European Environment Agency 2011; Environmental Protection Agency 2011).

Bioethanol is a renewable energy alternative to fossil fuels produced by fermentation of sugars using microorganisms. The complete combustion property of ethanol with reduced levels of carbon dioxide emissions can solve the global warming problem to some extent (Mc Kendry, P., 2002). It emits 85% less greenhouse gases compared to gasoline (Mete, A.M et al 2002) and till recently it has been produced mainly from crops but nowadays there is great interest in utilizing cheaper lignocellulosic materials as a feedstock for ethanol production. Moreover, bioethanol global scale production has been growing in the past few years due to some factors including environmental, social and energy security. (Ying Yang et al. 2009).

Lignocellulosic biomass mainly contains cellulose along with hemicelluloses and lignin. These feedstock's are the ideal renewable sources for ethanol production due to their high carbohydrate contents, relatively low cost, worldwide availability, and has little net production of greenhouse gases. (Ying Yang et al. 2009) (Liang Zhang et al 2012). Water hyacinth (Eichhornia crassipes) is one of the world's worst aquatic plants and it infests rivers, dams, lakes and canals all over the world. It grows very fast and completely covers entire water surface available which devastates aquatic environment and costs billions of dollars every year for its control (Burton, J., 2005). Currently this plant is utilized in the preparation of fish and livestock feeds, bio-gas production, charcoal, wastewater treatment for domestic and industrial use (Sotolu, A.O., 2012). In the current study it has been used for bioethanol production studies.

Lignocellulosic material has to be converted to simple fermentable sugars, through a hydrolysis process. There are several methods to lignocelluloses; among them the most popular are chemical and enzymatic hydrolysis methods (Ballesteros.et all 2008). Acid hydrolysis is easy and cheap method but produces by-products that are toxic to

yeast during fermentation. Enzymatic hydrolysis is time consuming, adds to the economics of ethanol production but sugar yields are specific and high with less or no inhibitory products formation (K. Srilekha Yadav et al 2011). Acid hydrolysis can be applied either as a pretreatment before enzymatic hydrolysis or can be used as a separate method for hydrolyzing lignocelluloses to fermentable sugars (Qureshi, N and Manderson, G., 1995).

In the present study both acid and enzymatic saccharification of the substrate was carried out for maximum fermentable sugar yield. In the first step, optimization of acid hydrolysis process parameters was carried out using dried and powdered substrate by varying acid concentration, temperature, time and biomass loading. In the second step, enzymatic hydrolysis of neutralized acid treated dried substrate was carried out to optimize process parameters by varying enzyme loading, hydrolysis time, temperature, agitation, surfactant and biomass loading.

2 MATERIALS AND METHODS

2.1 Raw materials

Fresh water hyacinth (*Eichhornia crassipes*) plants were harvested from local water bodies in Hyderabad, Andhra Pradesh, India. Extensive roots and rotten parts of the plants were discarded and the plants were washed with tap water to remove adhering dirt and then chopped and sun dried to about 55% moisture content. The partially dried sample was ground and dried at 65±3ºC until the final moisture content reached to less than 10%. The dried sample was then ground and sieved to a size of 350 µm. The grinder used in this experiment had the provision to fix a sieve with the desire pore size just before the outlet of sample such that the sample could be ground till it passes through the sieve. The ground sample was then packed in plastic bags.

2.2 Analysis of chemical composition of water hyacinth (Eichhornia crassipes)

The cellulose, lignin and hemicellulose fraction of water hyacinth (Eichhornia crassipes) were analyzed using standard TAPPI methods, 1992.

2.3 Acid and enzymatic hydrolysis

2.3.1 Acid hydrolysis

Five grams of powdered and dried native water hyacinth (Eichhornia crassipes) at 1:10 (solid: liquid) ratio was taken in 250 ml Erlenmeyer flask and was subjected to acid hydrolysis(sulphuric acid) with 1% acid concentration at temperature 110 ºC for 15 min. Later the contents were filtered with muslin cloth and the total reducing sugars in the filtrate were estimated after neutralizing the hydrolysate with 1N NaOH. The biomass was washed under tap water till neutral pH and dried to constant weight to use further for enzymatic saccharification.

2.3.2 Optimization of acid hydrolysis process parameters

Optimization of various parameters i.e. temperature, acid concentration, biomass loading and reaction time of acid hydrolysis was optimized in a stepwise procedure where specified parameter was varied by keeping all the other parameters constant. After hydrolysis in a particular process in each step the contents were filtered with muslin cloth and the filtrate was analyzed for sugars. The biomass was washed under tap water till neutral pH and dried to constant weight.

2.3.2.1Optimization of temperature

Effect of temperature on acid saccharification was carried out by varying temperature in the range between 50°C-130°C. A control at room temperature was also kept for comparison.

2.3.2.2 Optimization of acid concentration

To optimize acid saccharification, hydrolysis was carried out with different concentrations of acid in the range between 0.5% (v/v) - 5 % (v/v). A control with water was also kept for comparison.

2.3.2.3 Optimization of biomass loading

Effect of biomass loading on acid saccharification was carried out by loading biomass in different ranges between 1.5(w/v) - 2.5 (w/v).

2.3.2.4 Optimization of reaction time

Optimization of reaction time on acid saccharification was carried out by varying reaction time interval from 15min to 90min.

2.3.3 Enzymatic hydrolysis of acid hydrolyzed biomass

Enzymatic hydrolysis of acid hydrolyzed biomass after neutralization was carried out in 150 ml Erlenmeyer flask using Accelerase 1500 enzyme containing (cellulase 60 U/ml, xylanase 110 U/ml and β-glucosidase 55 U/ml) incubated at 150 rpm for 24h.Optimum temperature, 50°C and pH 5.5 were mentioned by the supplier. After incubation the samples were centrifuged and the supernatant was used to estimate amount of sugar released.

2.3.4 Optimization of enzymatic hydrolysis process parameters

Optimization of various parameters i.e. reaction time, agitation, biomass loading, surfactant, surfactant concentration and enzyme units was carried out in a stepwise procedure where specified parameter was varied by keeping all the other parameters constant. After hydrolysis in a particular process in each step the contents were centrifuged and the supernatant was used to estimate amount of sugar released.

2.3.4.1 Optimization of reaction time

Optimization of the enzymatic hydrolysis reaction time for maximum sugar released was done by collecting samples at different reaction time intervals starting from 6h to 98h at 50°C agitating at 150 rpm.

2.3.4.2 Optimization of agitation speed

Effect of agitation was carried out with different agitation with maximum speed upto 200 rpm. A control was also kept under static condition for reference.

2.3.4.3 Optimization of biomass loading

Effect of biomass loading was optimized by varying solid: liquid ratio between 0.5g to 3g. After incubation time the supernatant was used to estimate reduced sugars.

2.3.4.4 Selection of surfactant

To increase the release of sugars during enzymatic hydrolysis different surfactants like Tween-20, Tween-80, Triton X-100, Oleic acid and Glycerol were studied to select the one most effective among them. After incubation the supernatant was used to estimate reduced sugar. A control without any surfactant was also kept for reference.

2.3.4.5 Optimization of surfactant concentration

Optimization of surfactant concentration was carried after the selection of the best surfactant in different concentration ranges from 0.01 ml to 0.2 ml. A control without any surfactant was also kept for reference

2.3.4.7 Optimization of Enzyme units

Effect of Accelerate1500 enzyme was optimized by adding enzyme units in the range of 0.2 to 2ml in acetate buffer containing cellulase 60 U/ml, xylanase 110 U/ml and β-glucosidase 55 U/ml. A control without enzyme was also kept for reference.

2.4 Analytical methods.

The total reducing sugars present (in acid hydrolysis filtered & enzymatic hydrolysis supernatant) was estimated by the Dinitrosalicylic acid method of Miller, (1959).

2.5 Statistical analysis.

All the experiments were performed in triplicate and the results are presented as mean ± Standard deviation and one way ANOVA with Graphpad Prism6 demo software.

3. RESULT AND DISCUSSION

3.1 chemical composition of water hyacinth

The dried powder of water hyacinth was analyzed for chemical composition by using the TAPPI and compared with that of previous studies (Table 1). The dried powder of water hyacinth was found to contain 31.6±0.4% cellulose, 33.4±0.4% hemicellulose and 9.30±0.9 % lignin. The presence of cellulose and hemicellulose together make the total holocellulose content of 65±1.6%. The cellulose and hemicelluloses content of water hyacinth reported by other researchers are ranged from 17.8 to 34.19% and 17.66 to 49.2% respectively and the lignin content is ranged from 1.9 to 26.36%. This indicates that the composition of the water hyacinth varies by the place of growing environment.

Table1: chemical composition of water hyacinth of different sources

	Cellulose	Hemicellulose	lignin	Reference
1	18	33.39	26.36	Chanakya et all.1993
2	17.8	43.4	7.8	Patel et all 1993
3	35	18.3	1.9	Abraham and Kurup.,1996
4	18.4	49.2	3.55	Ashish Kumar a et ll 2009
5	34.19	17.66	12.22	Deuk Joo Ahn.et all.,2012
6	31.6±1.3	33.4±0.8	9.30±0.9	This study

3.2 Acid and enzymatic hydrolysis

3.2.1 Acid hydrolysis

Acid hydrolysis of powdered and dried 5g of water hyacinth (Eichhornia crassipes)was carried out at 1:10 (solid: liquid) ratio in 250 ml Erlenmeyer flask at temperature 110 ° C for 15 min and acid concentration of 1%.The amount of total reducing sugar released were estimated to be 190±9.5 mg/g.

3.2.2Acid hydrolysis process parameters optimization

To release maximum sugars from the substrate, optimization of acid hydrolysis parameters is required. After hydrolysis in a particular process in each step the contents were filtered with muslin cloth and the filtrate was analyzed for sugars. Results are presented in the Table2

3.2.2.1 Optimization of temperature

Increase in the hydrolysis temperature increased the sugar yield upto 120°C and further increase in temperature decreased the sugar yield. This variation may be due to degradation of sugar into furfural, hydroxyl methyl figures and other compounds at higher temperatures. The maximum sugar yield obtained at this temperature and acid concentration of 1% was about 220±8.8 mg/g of the substrate (table2). ANOVA analysis of data at P value 0.0035 with CI (confidence interval) of 95 %, (P?0. 05, R square 0.0024) shows a significant increase in reducing sugar yield with increase of temperature during acid hydrolysis. Ogawa et al., (2008) in his studies mentioned in conventional practice, high temperature and long time, increase level of total reducing sugar yield. In agreement to our studies Karri Satyanagalakshmi et al (2011) reported maximum sugar yield of 0.348±0.0014g/g reducing sugars at 121°C of temperature using 4% acid concentration for acid hydrolysis of water hyacinth.

3.2.2.2 Optimization of acid concentration

Increase in the concentration of acid during hydrolysis increased the sugar yield upto 2%v/v further increase in the concentration of acid decreased the sugar yield may be due to formation of inhibitory compounds due to rapid degradation of sugars at higher acid concentrations. The maximum amount of sugar released was 260±10.4 mg/g of the substrate (table2) by using 2% v/v sulphuric acid, at 120°C temperature with hydrolysis time of 15 minutes. ANOVA analysis of data at P value 0.001, CI (confidence interval) of 95 %, (P?0.05, or square 0.061) shows a significant increase in reducing sugar yield with increase of

acid concentration. Idrees et al (2012) carried out optimization of dilute acid pretreatment of water hyacinth biomass using sulphuric acid and reported 2% v/v concentration of acid as optimum for maximum sugar release from water hyacinth. Idrees et al carried out hydrolysis for 120 min by using 2% v/v sulphuric acid at 120ºC and obtained reducing sugars of 330±0.44 mg/g of the substrate

3.2.2.3 Optimization of biomass loading

Increase in the biomass loading during hydrolysis increased the sugar yield upto 1:10 w/v and further increase in the biomass loading decreased the sugar yield. The maximum sugar yield of 261±7.9 mg/g of the substrate was obtained by using 1:10 w/v biomass loading (table 2).Further increase of biomass loading decrease the sugar yield may be due insufficient reaction volume for converting biomass into sugar. ANOVA analysis of data on P value ? 0.001, CI (confidence interval) of 95 % (P ? 0.05, R square 0.2) shows a significant increase in reducing sugar yield with the increase of biomass loading during acid hydrolysis. P. Jeevan et al (2011) reported 1:10 ratio of solid: liquid as optimum level for their work on optimization studies on acid hydrolysis of corn cobs which supports our result. They optimized biomass loading for estimation of xylose, glucose and Arabinose after acid hydrolysis of Corn cob in three levels (1:6, 1:8 and1:10). They reported optimum loading at 1:10 with 70.9, 5.6 and 4.7 g/L for xylose, glucose and Arabinose.

3.2.2.4 Optimization of reaction time

Increasing the reaction time during hydrolysis increased the sugar yield up to 60 min further increase in the reaction time decreased the sugar yield. Maximum of 280±11.2 mg/g of reducing sugars were released from the substrate at 60 min reaction time interval during acid hydrolysis (table2). ANOVA analysis of data on P value ?0.001, CI (confidence interval) of 95 % (P?0.05, R Square 0.6) shows a significant increase in reducing sugar yield with increase of reaction time during acid hydrolysis. Ezeji et al (2007) reported that the less amount of reducing sugars were released at higher temperature and long residence time due to degradation of the xylose and arabinose into furfural which supports our work on optimization of reaction time which showed decrease in sugar release with long residence time of acid hydrolysis.

Table 2: Total reducing sugar yield after optimization of process parameter of acid hydrolysis of water hyacinth (Eichhornia crassipes)

Process parameters		Parameter range	TRD mg/g
Temperature			
	1	Room Temperature	2±0.12
	2	50 ºC	5±0.25
	3	80 ºC	30±1.35
	4	100 ºC	110±3.8
	5	110 ºC	190±9.5
	6	**120 ºC**	**220±8.8**
	7	130 ºC	215±7.5
Acid concentration			
	1	Water	24±1.2
	2	0.5%v/v	175±7
	3	1%v/v	215±8.6
	4	**2%v/v**	**260±10.4**
	5	3%v/v	255±10.2
	6	4%v/v	250±10
	7	5%v/v	245±9.8
Biomass loading			
	1	0.25:10w/v	240±7.2
	2	0.5:10w/v	244±5.3
	3	0.75:10w/v	250±6.4
	4	**1:10w/v**	**261±8.1**
	5	1.25:w/v	240±3.6
	6	1.5:w/v	240±4.4
Reaction time			
	1	15min	260±1.4
	2	30min	264±2.6
	3	45min	270±5.3
	4	**60min**	**280±3.4**
	5	90min	275±8.2

3.2.3 Enzymatic hydrolysis

Enzymatic hydrolysis of acid hydrolyzed biomass after neutralization was carried out in 150 ml Erlenmeyer flask using Accelerase 1500 enzyme containing mixture of cellulase 60 U/ml, xylanase 110 U/ml and β-glucosidase 55 U/ml. Hydrolysis was carried out for 24h and maximum of 260±8.3mg/g of total reducing sugar were released at 50 ºC and agitation at150 rpm.

3.2.4 Enzymatic hydrolysis process parameters optimization

To release maximum sugars from the substrate, optimization of enzymatic hydrolysis parameters is required. After hydrolysis in a particular process in each step the contents were centrifuged and the supernatant was used to estimate amount of sugar released. All results are presented in the Table 3

3.2.4.1 Optimization of reaction time

Enzymatic hydrolysis was carried out upto 96h and the samples were withdrawn for every 12h. The highest sugar yield of 290±5.4 mg/g was obtained at 48h of reaction time (Table3). Zhu L , et al (2008) reported due to irreversible adsorption of enzyme to the substrate, increase in the reaction time decreases the sugar yield. ANOVA analysis of the data at P value 0.01 with CI (confidence interval) of 95 %,

(P ? 0.05, F 94, or square 0.98) showed a significant increase in reducing sugar yield with increase in the reaction time during enzymatic hydrolysis.

3.2.4.2 Optimization of Agitation

Optimization of agitation during enzymatic hydrolysis was carried out by varying the agitation speed in the range between 50 rpm to 200 rpm. The higher yield of sugar i.e. 290±6.1 mg/g (Table3) was obtained at 150 rpm. Instability of enzyme at higher rpm decreases the amount of total reducing sugar yield with increase in agitation speed (Ravi Dhabhai, et al., 2012). ANOVA analysis at P value 0.0002 with CI (confidence interval) of 95 %, (P?0.05, F 3768, R square 1) results showed significant increase in reducing sugar yield with the increase agitation during enzymatic hydrolysis. Ravi Dhabhai, et al (2012) studies on different lignocellulosic materials (wheat straw, pearl straw and sugarcane bagasse) for production fermentable sugars by dilute acid pretreatment and enzymatic saccharification reported optimum agitation level as 170 rpm which is close to our result i.e.150 rpm and obtained total reducing sugars of 33.0 mg/g of the substrate.

3.2.4.3 Optimization of Biomass loading

Biomass loading was optimized by varying the amount of biomass in the reaction mixture. The amount of biomass varied between 0.5g to 3g. Biomass loading of 1g gave maximum reducing sugar concentration of 288±8.6 mg/g (Table3) of the substrate. Shanmugam & Sathishkumar 2009 in their studies mentioned for any enzyme mediated catalysis, the level of the reaction is correlated to the concentration of the substrate. Harikrishna et al (1998) reported at low substrate concentration the initial speed of the reaction is directly proportional and at high substrate concentration the speed of the reaction remains constant following a zero order reaction rate. Difficulties associated in mixing and end product inhibition decreased the sugar yield with further increase in biomass loading. In agreement to above conclusion the ANOVA analysis of data at P value 0.0003 with CI (confidence interval) of 95 %, (P?0.05, F 3333, R square 1) shows significant increase in reducing sugar yield with the increase of biomass loading during enzymatic hydrolysis.

3.2.4.4 Selection of the surfactant.

Surfactant process improves enzyme activity by reducing activity loss caused by non-productive adsorption (Zheng, Y et al (2008),), modifies the substrate surface property and decreases the irreversible binding of enzyme to substrate as reported by Qi et al (2010). Different types of surfactant were tried to enhance the enzymatic hydrolysis. As suggested the application of surfactant, among different types of surfactants studied Triton X-100 gave improved sugar yield of 317.9±7.4 mg/g (Table3) of the substrate. ANOVA analysis of the data at P value 0.0014 with CI (confidence interval) of 95 %, (P?0.05, F 71, R square 0.97) shows significant effect of surfactant on enzymatic hydrolysis.

2.4.5 Optimization of surfactant concentration

Optimization of surfactant concentration during enzymatic hydrolysis was carried out in the range between 0.02% (w/v) - 0.2% (w/v) of Triton X-100. Maximum total reducing sugars of 321.6±9.2 mg/g of substrate (Table3) were released at 0.1% (w/v) of Triton X-100; using 0.12% (w/v) and 0.14% (w/v) of surfactant also gave similar result. ANOVA analysis of data at P value 0.1549 with CI (confidence interval) of 95 %, (P?0.05, F 5, R square 0.71) showed no significant increase in reducing sugar yield with the increase of surfactant concentration during enzymatic hydrolysis. Karri Satyanagalakshmi et al (2011) reported Triton X-100 as best surfactant in the process of enzymatic saccharification and obtained 0.1% w/v of Triton X-100 as optimum level which supports our result.

3.2.4.5 Optimization of Enzyme units

Shanmugam & Sathishkumar., 2009 reported the enzyme activity is proportional to the amount of enzyme present only if other factors like temperature or pH are at optimum and do not affect the relationship between activity of catalysis and enzyme concentration. Therefore to investigate the effect of enzyme concentration on total reducing sugar yield in our experiments we carried enzymatic hydrolysis with different volume of Accelerate enzyme (0.25ml - 2ml). Maximum reducing sugar of 340±13.8 mg/g of the substrate (Table3) was released at 1ml of enzyme. ANOVA analysis of data at P value 0.0005 with CI (confidence interval) of 95 %, (P?0.05, F 20 78, R square 1) showed significant increase in reducing sugar yield with the increase the volume of enzyme during enzymatic hydrolysis

Table 3: Total reducing sugar yield in enzymatic process parameter optimization

Table 3: Total reducing sugar yield in enzymatic process parameter optimization			
Process parameters		Parameter range	TSR mg/g
Effect of reaction time on enzymatic hydrolysis			
	1	6h	250±7.7
	2	12h	260±5.3
	3	18h	270±9.4
	4	24h	270±9.7
	5	30h	275±7.1
	6	36h	278±6.7
	7	42h	280±8.6
	8	48h	280±10.2
	9	54h	285±8.5
	10	60h	285±8.8
	11	66h	288±6.2
	12	72h	**290±5.4**
	13	78h	288±8.9
	14	84h	285±10.3
	15	90h	280±8.6
	16	96h	280±7.3

Effect of different agitation on enzymatic hydrolysis			
	1	**STATIC**	18±2
	2	**50 RPM**	120±2.4
	3	**75 RPM**	160±3.3
	4	**100 RPM**	210±4.3
	5	**125 RPM**	250±5.2
	6	**150 RPM**	**290±6.1**
	7	**175 RPM**	220±4.5
	8	**200 RPM**	200±4.1
Effect of different biomass loading on enzymatic hydrolysis			
	1	**0.5g**	180±5.4
	2	**0.75g**	230±6.9
	3	**1g**	**288±8.6**
	4	**1.25g**	240±7.2
	5	**1.5g**	220±6.5
	6	**2g**	200±6
	7	**2.5g**	185±5.5
	8	**3g**	160±4.88
Effect of different types of Surfactant onenzymatic hydrolysis			
	1	**Control (no surfactant)**	279.16±7.7
	2	**TWEEN80**	303.1±8.6
	3	**TRITON**	**317.9±7.4**
	4	**TWEEN20**	290.2±11.8
	5	**OLIC ACID**	290.7±6.4
	6	**GLYCEROL**	297.7±6.2
Effect of surfactant concentration on enzymatic hydrolysis			
	1	**Control (no surfactant)**	275.16±4.6
	2	**0.02**	311.7±8.8
	3	**0.04**	317±6.2
	4	**0.06**	318.2±6.9
	5	**0.08**	317.4±7.8
	6	**0.1**	**321.6±9.2**
	7	**0.12**	314.6±8.9
	8	**0.14**	313.8±7.4
	9	**0.16**	312.1±10.1
	10	**0.18**	311±9.2
	11	**0.2**	310.2±13.2
Figure 2F: Effect of enzyme volume on enzymatic hydrolysis			
	1	**No enzyme**	0.0
	2	**0.25ml**	250±9.5
	3	**0.5ml**	320±12.2
	4	**0.75ml**	330±12.5
	5	**1ml**	**340±13.8**
	6	**1.5ml**	300±11
	7	**2ml**	260±9.8

4.0 CONCLUSION

The lignocellulosic substrate water hyacinth (Eichhornia crassipes) with holocellulose content of 65% was subject to acid (H_2SO_4) and then enzyme (Accelerate 1500) saccharification .Table 4 shows optimum condition of acid and enzyme hydrolysis steps .we found maximum total reducing sugar of 280±11.2 mg/g of the substrate for acid hydrolysis at temperature (120°C), acid concentration (2%v/v), biomass loading (1:10w/v) and time (90min). Data on enzymatic hydrolysis obtained maximum total reducing sugar of 340±13.8mg/g at time 72h, temperature 50°C, agitation 150RPM, biomass loading 1g, surfactant (TR ITON), surfactant concentration 0.1% and enzyme volume 1ml.The total saccharification of water hyacinth through acid & enzyme saccharification obtained is around 95.40%. Therefore, water hyacinth seems to be a potential biomass as a low cost source for fermentable sugars and bio ethanol, xylitol production at industrial level.

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