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Pretreatment Studies of Cellulose Wastes for Optimization of Cellulase Enzyme Activity

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ABSTRACT

The composition of cellulose, hemicellulose, starch, lignin, pectin, protein, and total lipid content in the selected cellulosic wastes-tapioca *(Manihot esculenta)* stem, leaf, petiole, and water hyacinth *(Eichhornia crassipes)* were determined. The effectiveness of various physical and chemical pretreatments on the enzymatic digestibility of these wastes were identified. In general, chemical pretreatments were more effective than physical pretreatments. The efficiency of the pretreatment was checked by subjecting these wastes to enzymatic saccharification after the pretreatments.

Index Entries: Cellulase; cellulose; pretreatment; bioconversion.

INTRODUCTION

The conversion of agricultural residues into fuel has been receiving increasing attention in recent years. The upward spiral in the price of fossil fuel, coupled with impending shortages, has made agricultural residues an attractive commodity as a supplemental source of energy. Tapioca waste and water hyacinth represent a renewable and low-cost energy resource and is available in significant amounts in Kerala.

Enzymatic hydrolysis of native lignocellulosics is generally a slow process. The resistance of biomass to enzymatic attack can be attributed to the following three major factors *(1–3)*:

1. Cellulose in lignocellulosic biomass possesses highly resistant crystalline structure;
2. Lignin surrounding cellulose forms a physical barrier;
3. Sites available for enzymatic attack are limited.

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Cellulose in lignocellulosics is composed of crystalline and amorphous components. The amorphous component is digested more easily by enzymatic attack than the crystalline component, and any means that will increase the amorphous content will enhance the hydrolysis rate. The presence of lignin forms a physical barrier for enzymatic attack, and hence, pretreatments causing disruption of the lignin linkage, increase the accessibility of cellulose and eventually its hydrolysis rate. The limitation of available sites for enzymatic attack seems from the fact that the average size of the capillaries in biomass is too small to allow the entry of large enzyme molecules, and the enzymatic attack is confined, therefore, to the external surface.

Thus, pretreatment is an essential prerequisite to enhance the susceptibility of lignocellulosic residues to enzyme action. An ideal pretreatment would accomplish reduction in crystallinity, concomitant with a reduction in lignin content and an increase in surface area. Many different pretreatments have been attempted *(4–7)*. The pretreatment can be divided into physical and chemical, depending on their modes of action on the substrate.

Physical pretreatments can be grouped into mechanical and nonmechanical pretreatments *(8,9)*. Chemical pretreatments have been used extensively to remove lignin and structural modifications of lignocellulosics.

It appears that most research efforts on pretreatment for enzymatic hydrolysis have been focused on pure cellulose, whereas little attention has been paid to lignocellulosics. In the present study, tapioca waste and water hyacinth were subjected to various physical and chemical pretreatments and evaluated the effect of these pretreatments on the enzymatic digestibility. This study will help to identify effective pretreatments and the relative importance of individual pretreatments on the rate of enzymatic hydrolysis of the lignocellulosics.

MATERIALS AND METHODS

Samples and Sampling

Tapioca waste-stem, leaf, and petiole was collected from the cultivation fields and the fresh water weed-water hyacinth was collected from the backwaters in Kerala. All the samples were dried at 60°C and powdered.

Composition of the Sample

From each of the samples, cellulose content was estimated by the method of Updegraft *(10)*. Hemicellulose content was estimated by the method of Goering and Van Socst *(11)*. Starch content was estimated by the method of Hodge and Hofreiter *(12)*. Pectin content was estimated by the method of Ranganna *(13)*. Lignin content was estimated by the method of Chesson *(14)*. Protein content was estimated by the method of Lowry et al. *(15)*. Total lipid content was estimated using a mixture of chloroform and methyl alcohol *(16)*.

Pretreatment Studies of Cellulosic Wastes

Since the sample contained appreciable amount of lipids and proteins, the samples were defatted with petroleum ether (60–80°C) in a soxlet extraction unit. The lipid free substance was subjected to papain digestion *(17)*. After complete hydrolysis, the protein content was analyzed and it was found that there was no detectable protein in the sample. The sample was dried, powdered and the powder was used for the following studies.

Physical Pretreatment

Physical pretreatment of tapioca waste and water hyacinth was carried out by milling and steaming.

Chemical Pretreatment

Sodium Hydroxide Pretreatment

Sodium hydroxide pretreatment was performed with 1,2,4,6,8, and 10% NaOH solution. Ten grams each of cellulosic wastes were mixed with 100 mL each of the NaOH solution separately and allowed to stand at room temperature for 2 h.

Sodium Hydroxide-Acetic Acid Pretreatment

In this method 10 g each of cellulosic wastes was mixed with a mixture of 100 mL of 1% NaOH and 2% acetic acid (1:1) separately and incubated at room temperature for 10 h.

Chloroform Pretreatment

In this pretreatment, 10 g each of cellulosic wastes was mixed with 100 mL of chloroform separately at room temperature for 3 h.

Acid Pretreatment

Acid pretreatment of cellulosic wastes was carried out by using dilute hydrochloric acid. Ten grams each of cellulosic wastes was soaked in 100 mL of 3% HCl separately at room temperature for 3h.

Sodium Sulphite Pretreatment

In sodium sulphite pretreatment, 13.7% sodium sulphite solution was used. Ten grams each of the cellulosic wastes were soaked in 70 mL of sodium sulphite solution separately. The mixture was allowed to stand for 2 h at room temperature.

Peracetic Acid Pretreatment

A solution of 1:1 v/v acetic anhydride and 35% hydrogen peroxide was used for peracetic acid pretreatment. Ten grams each of the cellulosic wastes were boiled with 100 mL of peracetic acid at 100°C for 30 min.

Butanol Pretreatment

A solution of 1:1 v/v butanol and water was used for butanol pretreatment. Ten grams each of the cellulosic wastes were kept at 121°C with 100 mL of the pretreating reagent separately in the presence of a 0.005% aluminum chloride as catalyst for 2 h.

Hydrogen Peroxide-Ferrous Salt Pretreatment

A solution of 100 mL of 1% hydrogen peroxide containing 100 mg of ferrous salt was used for hydrogen peroxide-ferrous salt pretreatment. Ten grams each of the cellulosic wastes were soaked in 100 mL of the pretreating reagent separately and kept at room temperature for 10 h.

Hydrogen Peroxide-Manganous Salt Pretreatment

A solution of 100 mL of 1% hydrogen peroxide containing 100 mg of manganous salt was used for hydrogen peroxide-manganous salt pretreatment. Ten grams each of the cellulosic wastes were immersed in 100 mL of the pretreating reagent separately and kept at room temperature for 10 h.

Hydrochloric Acid-Zinc Chloride Pretreatment

A solution of 100 mL of 2% hydrochloric acid containing 100 mg of zinc chloride was used for hydrochloric acid-zinc chloride pretreatment. Ten grams each of the cellulosic wastes were treated with 100 mL of the pretreating reagent separately and kept at room temperature for 10 h.

Acetic Acid-Hydrogen Peroxide Pretreatment

A solution of 1:1 v/v 1% hydrogen peroxide and 1% acetic acid was used for acetic acid-hydrogen peroxide pretreatment. Ten grams each of the cellulosic wastes were treated separately with 100 mL of the pretreating reagent and kept at room temperature for 10 h.

After physical pretreatments, the finely powdered samples of all the cellulosic wastes were washed with distilled water to remove all the soluble contents present in the samples. The washing with water was continued until the wash water was clear. After chemical pretreatments, the various samples of cellulosic wastes were washed with distilled water until the wash water was neutral. Dried overnight at 80°C and used as the sample for saccharification studies.

Enzymatic Saccharification Of Cellulose Waste

Enzymatic hydrolysis of each of the pretreated samples was carried out using cellulase enzyme from each of the different microorganisms *M. verrucaria, C. comatus, P. florida* and *Cellulomonas* sp. The enzyme solution was prepared in our laboratory as mentioned earlier *(18)* and the saccharification was carried out by the method of Johnson et al. *(19)*. The saccharification efficiency was checked by measuring the amount of reducing sugar formed per 100 mg substrate per mg protein.

Table 1
Composition of Materials

Samples	Cellulose	Hemicellulose	Lignin	Starch	Pectin	Proteins	Lipids
Tapioca stem	42.20 ± 0.82	16.45 ± 0.27	15.50 ± 0.26	2.13 ± 0.04	0.25 ± 0.002	11.40 ± 0.25	9.30 ± 0.15
Tapioca petiole	37.60 ± 0.72	24.55 ± 0.48	10.36 ± 0.19	2.56 ± 0.02	0.19 ± 0.003	10.20 ± 0.22	6.00 ± 0.10
Tapioca leaf	18.45 ± 0.46	15.40 ± 0.34	3.12 ± 0.08	4.36 ± 0.03	0.18 ± 0.001	22.00 ± 0.48	16.00 ± 0.38
Water hyacinth	35.02 ± 0.77	18.32 ± 0.38	4.60 ± 0.11	1.85 ± 0.01	0.08 ± 0.001	11.20 ± 0.20	8.01 ± 0.15

Average of five values in each case ± SEM.
Values are expressed as mg/100 mg sample.

RESULTS AND DISCUSSION

Composition

Cellulose, hemicellulose, lignin, starch, pectin, proteins and total lipids present in the samples were estimated and the results are given in Table 1. Cellulose and lignin contents were found to be more in tapioca stem. Hemicellulose was maximum in tapioca petiole. But there was not much difference in hemicellulose content in tapioca stem and petiole. On the other hand, tapioca leaves were rich in proteins and lipids. Proteins and lipids were found to be less in tapioca petiole. Tapioca stem contained maximum pectin content followed by tapioca petiole, leaf, and water hyacinth. On the other hand, starch content was highest in tapioca leaf followed by petiole, stem, and water hyacinth.

Pretreatment Studies

The effect of pretreatments on tapioca stem are given in Table 2. Among the various pretreatments, 2% NaOH was found to be the most suitable pretreatment agent for tapioca stem. The 2% NaOH-pretreated tapioca stem gave the maximum saccharification with the enzymes from four different organisms. *M. verrucaria* cellulase gave the maximum saccharification followed by *C. comatus, P. florida* and *Cellulomonas sp.* But as the nature of the pretreatment varies, the efficiency of cellulosic wastes to saccharification varies, as in turn with the enzyme source. Following 2% NaOH, NaOH-CH_3COOH, 4% NaOH and 6% NaOH were found to be the good pretreatment agents for tapioca stem when the saccharification was carried out with *M. verrucaria* cellulase. 1% NaOH and HCl; 8% NaOH and

Table 2
Effect of Pretreatments on Tapioca Stem

Pretreatment	Source of enzyme			
	M. v.	C. c.	P. f.	Cell.
Physical pretreatments				
Milling	7.75 ± 0.06	5.75 ± 0.04	5.00 ± 0.06	3.50 ± 0.07
Steaming	8.50 ± 0.10	6.06 ± 0.13	5.36 ± 0.10	3.90 ± 0.05
Chemical pretreatments				
1% NaOH	17.00 ± 0.20	13.74 ± 0.12	12.15 ± 0.10	8.82 ± 0.52
2% NaOH	19.88 ± 0.11	16.09 ± 0.15	14.95 ± 0.21	10.19 ± 0.28
4% NaOH	18.02 ± 0.10	13.05 ± 0.07	11.07 ± 0.13	6.25 ± 0.05
6% NaOH	17.20 ± 0.15	12.56 ± 0.07	10.38 ± 0.12	5.57 ± 0.04
8% NaOH	15.07 ± 0.20	11.72 ± 0.10	9.63 ± 0.09	5.12 ± 0.01
10% NaOH	12.37 ± 0.20	8.85 ± 0.10	8.71 ± 0.20	4.30 ± 0.13
NaOH-CH_3 COOH	18.90 ± 0.17	13.09 ± 0.26	12.25 ± 0.20	8.13 ± 0.07
$CHCl_3$	13.94 ± 0.20	10.98 ± 0.25	9.96 ± 0.10	4.78 ± 0.04
HCl	16.03 ± 0.25	13.09 ± 0.18	12.69 ± 0.15	7.83 ± 0.10
Na_2SO_3	7.52 ± 0.19	5.86 ± 0.10	5.13 ± 0.04	3.55 ± 0.05
Peracetic acid	15.04 ± 0.19	12.56 ± 0.17	12.17 ± 0.10	5.03 ± 0.05
Butanol	8.10 ± 0.10	6.52 ± 0.04	6.11 ± 0.10	5.42 ± 0.20
H_2O_2-Fe^{2+}	7.56 ± 0.10	6.22 ± 0.19	5.62 ± 0.13	3.94 ± 0.09
H_2O_2-Mn^{2+}	13.67 ± 0.19	11.45 ± 0.15	10.23 ± 0.09	4.87 ± 0.06
HCl-$ZnCl_2$	15.62 ± 0.19	12.59 ± 0.10	11.13 ± 0.16	7.18 ± 0.09
CH_3COOH- H_2O_2	14.27 ± 0.20	12.87 ± 0.15	12.05 ± 0.13	8.00 ± 0.10

Average of 5 values in each case ± SEM
Values are expressed as mg of reducing sugar per 100 mg substrate per mg protein
M. v. - M.verrucaria
C. c. - C. comatus
P. f. - P. florida
Cell. - Cellulomonas sp.

peracetic acid; $CHCl_3$ and H_2O_2-Mn^{2+} have the same pretreatment efficiency on tapioca stem with *M. verrucaria* cellulase. Using this enzyme, pretreatment by steaming was better than H_2O_2-Fe^{2+}, butanol and Na_2SO_3 pretreatments.

For *C. comatus* enzyme, 1% NaOH was the second best pretreatment agent for tapioca stem. When the saccharification was done by *C. comatus* cellulase, it was found that HCl and NaOH-CH_3COOH pretreatment have the same efficiency on tapioca stem. In the case of cellulase from *P. florida*, besides 2% NaOH, the best pretreatment can be done with HCl, followed by NaOH-CH_3COOH (besides 2% NaOH). The pretreatment carried out by peracetic acid and 1% NaOH; HCl-Zn^{2+} and 4% NaOH gave the same saccharification efficiency using this enzyme. NaOH (1%) pretreatment can

Table 3
Effect of Pretreatments on Tapioca Petiole

Pretreatment	Source of enzyme			
	M.v.	C.c.	P.f.	Cell.
Physical pretreatments				
Milling	7.32 ± 0.01	5.32 ± 0.01	4.80 ± 0.03	3.30 ± 0.06
Steaming	7.50 ± 0.06	5.91 ± 0.10	5.10 ± 0.08	3.75 ± 0.06
Chemical pretreatments				
1% NaOH	16.10 ± 0.19	12.59 ± 0.07	11.13 ± 0.06	8.12 ± 0.40
2% NaOH	11.35 ± 0.06	9.16 ± 0.03	9.00 ± 0.10	4.41 ± 0.01
4% NaOH	10.58 ± 0.05	8.90 ± 0.15	8.02 ± 0.09	4.20 ± 0.02
6% NaOH	10.12 ± 0.06	7.80 ± 0.07	7.62 ± 0.13	3.62 ± 0.06
8% NaOH	7.54 ± 0.09	6.52 ± 0.08	5.26 ± 0.03	2.87 ± 0.02
10% NaOH	6.73 ± 0.05	6.22 ± 0.09	5.22 ± 0.11	2.60 ± 0.05
NaOH-CH_3 COOH	10.87 ± 0.10	8.64 ± 0.09	8.04 ± 0.10	3.95 ± 0.01
$CHCl_3$	12.53 ± 0.18	10.30 ± 0.17	9.06 ± 0.13	4.61 ± 0.03
HCl	10.62 ± 0.19	7.94 ± 0.08	7.70 ± 0.20	4.40 ± 0.01
Na_2SO_3	7.38 ± 0.17	5.81 ± 0.13	5.10 ± 0.03	3.53 ± 0.10
Peracetic acid	18.22 ± 0.25	14.88 ± 0.15	13.17 ± 0.09	9.95 ± 0.09
Butanol	7.04 ± 0.09	6.24 ± 0.09	5.54 ± 0.13	4.01 ± 0.17
H_2O_2-Fe^{2+}	7.60 ± 0.08	6.52 ± 0.15	5.74 ± 0.14	4.29 ± 0.06
H_2O_2-Mn^{2+}	8.52 ± 0.08	6.87 ± 0.17	6.05 ± 0.07	3.00 ± 0.01
HCl-$ZnCl_2$	9.86 ± 0.05	8.05 ± 0.20	7.17 ± 0.13	5.26 ± 0.01
CH_3COOH- H_2O_2	13.49 ± 0.10	11.11 ± 0.10	9.22 ± 0.15	7.07 ± 0.11

Average of 5 values in each case ± SEM
Values are expressed as mg of reducing sugar per 100 mg substrate per mg protein
M. v. - M.verrucaria
C. c. - C. comatus
P. f. - P. florida
Cell. - Cellulomonas sp.

make tapioca stem to a better substrate for the bacterial enzyme besides 2% NaOH. In all the four cases, it was found that physical pretreatments had very less influence on tapioca stem than the chemical pretreatment.

The results of the effect of pretreatments on tapioca petiole are given in Table 3. Whatever may be the enzyme source, peracetic acid followed by 1% NaOH and CH_3COOH-H_2O_2 pretreatment makes this cellulosic waste more susceptible to enzymatic saccharification. When the pretreatment was carried out by different reagents, it was found that the rate of saccharification varies with the enzyme source. H_2O_2-Fe^{2+} and 8% NaOH pretreatments gave the same saccharification rate with the enzyme from *M. verrucaria* and *C. comatus*. On the other hand, NaOH-CH_3COOH and 4% NaOH as well as HCl and 2% NaOH pretreatments could provide the

Table 4
Effect of Pretreatments on Tapioca Leaf

Pretreatment	Source of enzyme			
	M.v.	C.c.	P.f.	Cell.
Physical pretreatments				
Milling	5.20 ± 0.05	4.09 ± 0.02	3.54 ± 0.01	2.48 ± 0.03
Steaming	5.34 ± 0.04	4.30 ± 0.09	3.80 ± 0.04	2.63 ± 0.01
Chemical pretreatments				
1% NaOH	12.13 ± 0.07	10.12 ± 0.05	9.12 ± 0.05	6.61 ± 0.41
2% NaOH	11.36 ± 0.10	10.30 ± 0.20	10.12 ± 0.05	7.35 ± 0.07
4% NaOH	12.42 ± 0.07	11.07 ± 0.01	10.37 ± 0.09	7.45 ± 0.04
6% NaOH	13.29 ± 0.10	11.72 ± 0.09	10.60 ± 0.15	7.52 ± 0.11
8% NaOH	15.17 ± 0.18	13.03 ± 0.15	12.18 ± 0.18	7.93 ± 0.07
10% NaOH	16.98 ± 0.23	14.87 ± 0.18	13.38 ± 0.19	7.98 ± 0.01
NaOH-CH_3 COOH	11.04 ± 0.13	9.56 ± 0.17	9.39 ± 0.12	6.25 ± 0.05
$CHCl_3$	15.62 ± 0.11	13.29 ± 0.18	12.05 ± 0.19	7.97 ± 0.07
HCl	11.09 ± 0.06	10.07 ± 0.21	9.92 ± 0.09	6.46 ± 0.09
Na_2SO_3	4.97 ± 0.08	4.13 ± 0.01	3.73 ± 0.10	2.33 ± 0.01
Peracetic acid	16.68 ± 0.13	14.35 ± 0.19	10.88 ± 0.11	7.39 ± 0.06
Butanol	5.88 ± 0.05	4.53 ± 0.10	4.05 ± 0.05	2.93 ± 0.03
H_2O_2-Fe^{2+}	5.92 ± 0.01	4.77 ± 0.08	4.46 ± 0.18	3.12 ± 0.04
H_2O_2-Mn^{2+}	10.06 ± 0.11	9.07 ± 0.13	8.02 ± 0.01	4.20 ± 0.02
HCl-$ZnCl_2$	12.00 ± 0.10	10.52 ± 0.18	10.06 ± 0.10	5.62 ± 0.05
CH_3COOH- H_2O_2	6.39 ± 0.09	5.26 ± 0.09	5.06 ± 0.20	3.72 ± 0.15

Average of 5 values in each case ± SEM
Values are expressed as mg of reducing sugar per 100 mg substrate per mg protein
M. v. - M.verrucaria
C. c. - C. comatus
P. f. - P. florida
Cell. - Cellulomonas sp.

same saccharification rate for the enzyme from *P. florida* and *Cellulomonas* sp. respectively. Irrespective of the enzyme source, pretreatment with 10% NaOH makes the tapioca petiole very less susceptible to enzyme attack. Here also chemical pretreatments predominates over the physical pretreatments.

The results of the effect of pretreatments on tapioca leaf are given in Table 4. Ten percent NaOH followed by peracetic acid and $CHCl_3$ pretreatments, made tapioca leaf easily susceptible to enzymatic attack with *M. verrucaria* and *C. comatus* cellulase. But 10% NaOH followed by 8% NaOH and $CHCl_3$ pretreatments were found to be suitable for this cellulosic waste with *P. florida* and *Cellulomonas* sp. cellulase. Both physical and sodium sulphite pretreatments have very less influence on tapioca leaf.

Table 5
Effect of Pretreatments on Water Hyacinth

Pretreatment	Source of enzyme			
	M.v.	C.c.	P.f.	Cell.
Physical pretreatments				
Milling	6.60 ± 0.02	5.06 ± 0.05	4.64 ± 0.02	3.26 ± 0.05
Steaming	6.88 ± 0.06	5.63 ± 0.21	4.97 ± 0.05	3.56 ± 0.03
Chemical pretreatments				
1% NaOH	9.94 ± 0.02	8.01 ± 0.01	7.08 ± 0.03	4.41 ± 0.24
2% NaOH	8.52 ± 0.05	6.87 ± 0.18	6.07 ± 0.09	3.73 ± 0.08
4% NaOH	8.27 ± 0.04	7.57 ± 0.06	6.02 ± 0.05	3.45 ± 0.02
6% NaOH	9.30 ± 0.05	9.11 ± 0.05	7.56 ± 0.14	6.30 ± 0.09
8% NaOH	8.72 ± 0.15	8.34 ± 0.10	6.83 ± 0.05	5.81 ± 0.05
10% NaOH	8.03 ± 0.07	6.54 ± 0.15	6.03 ± 0.05	5.00 ± 0.04
NaOH-CH_3 COOH	10.45 ± 0.08	9.03 ± 0.16	7.52 ± 0.08	4.87 ± 0.09
$CHCl_3$	10.88 ± 0.09	9.66 ± 0.05	7.79 ± 0.04	5.00 ± 0.01
HCl	12.43 ± 0.10	11.15 ± 0.03	10.17 ± 0.05	6.85 ± 0.13
Na_2SO_3	6.63 ± 0.03	5.40 ± 0.04	4.63 ± 0.11	3.31 ± 0.02
Peracetic acid	18.54 ± 0.11	15.13 ± 0.18	13.62 ± 0.15	10.10 ± 0.09
Butanol	7.60 ± 0.01	6.41 ± 0.01	5.60 ± 0.04	5.10 ± 0.10
H_2O_2-Fe^{2+}	7.61 ± 0.05	6.57 ± 0.11	5.80 ± 0.06	4.47 ± 0.08
H_2O_2-Mn^{2+}	9.94 ± 0.13	8.01 ± 0.09	7.08 ± 0.05	3.97 ± 0.10
HCl-$ZnCl_2$	8.52 ± 0.08	6.91 ± 0.05	6.07 ± 0.06	3.64 ± 0.07
CH_3COOH- H_2O_2	11.36 ± 0.17	9.35 ± 0.17	8.10 ± 0.21	6.02 ± 0.03

Average of 5 values in each case ± SEM
Values are expressed as mg of reducing sugar per 100 mg substrate per mg protein
M. v. - M.verrucaria
C. c. - C. comatus
P. f. - P. florida
Cell. - Cellulomonas sp.

The enzymatic saccharification of this waste was maximum with the enzyme from *M. verrucaria* and least with that of *Cellulomonas* sp.

The results of the effect of pretreatments on water hyacinth are given in Table 5. Among the various pretreatments, peracetic acid followed by HCl pretreatment was found to be the most suitable for water hyacinth, irrespective of the enzyme source. CH_3COOH-H_2O_2 and $CHCl_3$ pretreatments can also be recommended for water hyacinth. Alkali pretreatments had not significant influence on water hyacinth. H_2O_2-Fe^{2+}, butanol and Na_2SO_3 pretreatments are not good for water hyacinth. In this case also cellulase from *M. verrucaria* showed the maximum saccharification rate and was followed by *C. comatus*, *P. florida*, and *Cellulomonas* sp.

CONCLUSION

The solid wastes of tapioca plant and water hyacinth consist mainly cellulose and lignin in considerable quantity. The physico-chemical properties of lignocellulosic wastes determined the rate of enzymatic degradation of this cellulosic wastes. Both cellulose and hemicellulose as such are highly susceptible to the action of cellulolytic microbes. But in nature it exists as a complex with lignin. Therefore liberation of cellulose and hemicellulose moiety either by chemical or enzymic reaction was reported to be essential for bioconversion. For different cellulosic wastes, different methods of pretreatments were suggested. Studies have shown that both physical and chemical pretreatments had some influence on saccharification rate.

The physical pretreatment, milling, reduced the particle size and provided more surface area for the action of enzyme. The difference in the rate of saccharification with the enzymes from different organisms might be due to the differences in the various fractions of cellulase enzyme as indicated by Dunlap and Paul *(20,21)*. The enhanced rate of saccharification by enzymes of various microorganisms after steaming may be due to the swelling of cellulosic material.

The chemical pretreatment carried out by using alkali increased the saccharification rate of cellulosic wastes. This might be due to the increase in the fibre saturation point and the swelling capacity of lignocellulosic materials. The increase in swelling capacity results from the saponification of esters of 4-0-methyl glucuronic acid attached to xylan chains. In the natural state, the esters act as crosslinks, limiting the swelling or dispersion of polymer segments in water. In the present study, pretreatment with alkali was found to be the most suitable one for tapioca stem and leaf. For water hyacinth and tapioca petiole, pretreatment carried out by using peracetic acid was found to be more suitable than alkali pretreatment. This might be due to their difference in composition and structure.

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