working wavelength for the determination of acetaminophen, because this point is the zero-crossing wavelength of codeine. The calculated and measured concentrations of acetaminophen at the optimum working wavelength are in very good agreement (Tables 1 and 2).

REFERENCES

1. Plakogiannis F.M. and Saad A.M.; **J. Pharm. Sci.**, 1975, 64; 1547.
2. Lotfi E.A.; **Can. J. Pharm. Sci.**, 1980, 15, 1191.
3. Carnevale C.; **J. Pharm. Sci.**, 1983, 72, 196.
4. Srivastava M.K., Ahmad S., Singh D. and Shukla I.C.; **Analyst.**, 1985, 110; 735.
5. Mahgoub H., **Drug Dev. Ind. Pharm.**, 1990, 16, 1011.
6. Milch G. and Szabo E; **J. Pharm. Biomed. Anal.**, 1991, 9, 1107.

Saccharification Studies of Lignocellulosic Biomass from Antigonum Leptopus Linn

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The ability of Trichoderma reesei QM-9414 cellulose complex to hydrolyse lignocellulosic biomass of Antigonum leptopus Linn was studied. Alkaline H_2O_2 pretreatment; 50°, pH 4.5, cellulose, 40 FPU/g substrate and substrate 2.5% were found to be optimum. Reaction time was reasonably less (24 h) with A. leptopus leaves compared with other substrates (48 h and more) due to the fine microsrystalline cellulose present in the leaves of A. leptopus.

CELLULOSE is an important renewable raw material produced in large amounts by plants.[1] Enzymatic saccharification of cellulose to produce sugars, which can later be transformed to chemicals or fuels is considered as a biotechnological process with enormous potential[2]. So far, significant progress was made using materials like saw dust,[3] News paper[4], tissue paper,[5] sugarcane bagasse,[6] wood substrates[7] and straw[8]. Studies were also performed on lignocellulose of **Onopordum nervosum** (Bioss)[2]. Screening suitable substrates from various lignocelluloses is important for designing an economically feasible process. By the initial studies carried out to determine new sources, we the initial studies carried out to determine new sources, we identified a new lignocellulosic material **A. leptopus** Linn, a weedy creeper, which is abundatly available in almost all parts of India and has not been exploited commercially. The crystallinity of the cellulose present in its leaves was found to be very less compared to other raw materials. Transforming this biomass would permit not only a new source of energy and chemicals but also give good yield in a shorter time.

T. reesei QM-9414 (NCIM 1186) was procured from NCL, Pune. Cellulose production and partial purification were carried out as per Mandels **et al** (1976)[9].

A. leptopus leaves were collected in Andhra University area, Visakhapatanam. Sun-dried leaves

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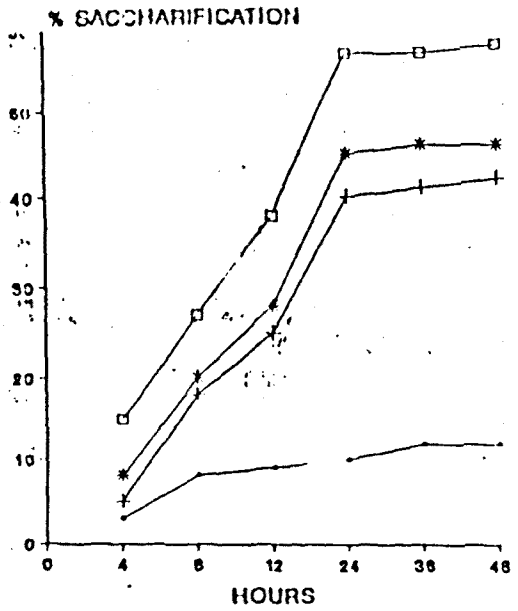

Fig. 1 C: Effect of Pretreatments on Saccharification L : 2.5% Substrate, 8 FPU Cellulase, 50°C, 4.5 pH. Untreated (•---•), Autoclaved (+—+) Alkali treated (*--*), Alkaline H_2O_2 treated (□--□)

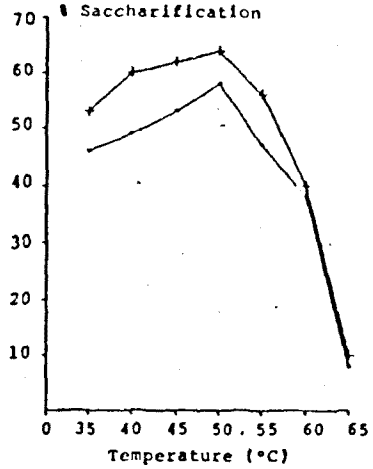

Fig. 2 C: Effect of Temperature on Saccharification L : 2.5% Substrate, 8 FPU Cellulase, 4.8 pH, 48 h. A. leptopus (•---•), Sugarcane (+—+)

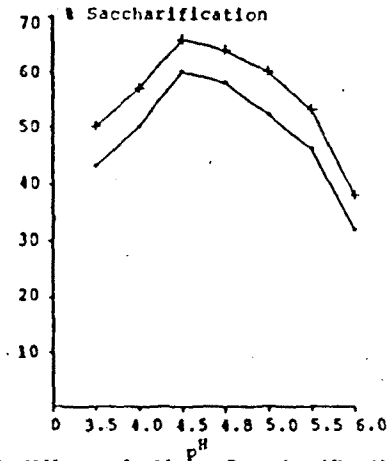

Fig.3 C: Effect of pH on Saccharification L: 2.5% Subsrate, 8 FPU Cellulose, 50°C, 48 h. A. leptopus (.•---•.), Sugarcane (+—+)

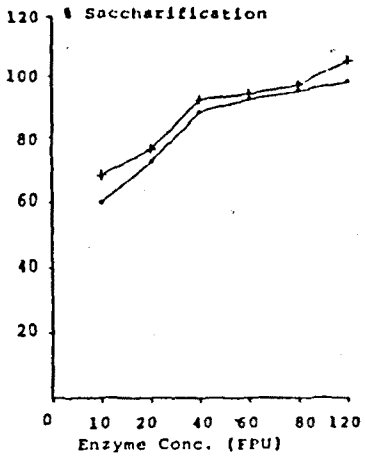

Fig 4. C: Effect of Enzyme concentration on Saccharification L : 2.5% Substrate, 50°C, 4.5 pH, 48h. A.leptopus (•—•), Sugarcane (+—+)

were used throughout. Untreated substrate was small pieces of leaves soaked in distilled water to remove soluble materials and dried. The substrate (500 g) was autoclared for 20 min at 15 lb pressure and the dried in an oven for 1 h to reduce moisture content to 40-50%. Substrate was then pulverized and sieved through U.S. standard screens (No. 20-0.84 mm particle size). Alkali cooking was done by cutting the leaves (20g) into 2-3 cm pieces and autoclaving with 360 ml NaOH (1.0%) solution at 120° for 2 h in a 1000 ml Erlenmeyer flask. After squeezing the cellulosic waste with nylon cloth, residual substrate was thoroughly washed with water followed by air drying at 45°. Alkaline H_2O_2 treatment was carried out as per Gould and Freer (1984)[10]. Saccharification experiments were carried out in stoppered flasks (100 ml) in presence of 0.01% sodium azide. To the pretreated substrate (2.5%) was added the cellulose (8 FPU/g substrate) in a total volume of 50 ml. The flasks were incubated on a rotary shaker at 150 rpm. Samples were taken periodically, centrifuged and supernatents were analysed for reducing sugars by DNS (Dinitro salicylic acid) method[11]. Cellulose activity (FPU, Filter Paper Units) was measured as per Mandels et al. (1976)[9].

Table 1 : Periodical Saccharrification Analysis

Optimum Parameter	Other Conditions	% Saccharification			
		A. leptopus		Sugarcane	
		24 h	48 h	24 h	48 h
50°	2.5% Subsrate, pH 4.8 Cellulose 8 FPU	57	58	55	64
pH 4.5	2.5% Subsrate, 50°, Cellulase 8 FPU.	58	60	55	66
50 FPU Cellulase	2.5% Subsrate, 50°, pH 4.5	86	88	76	92
2.5% Subsrate	Cellulase 40 FPU pH 4.5, 50°	86	88	76	92

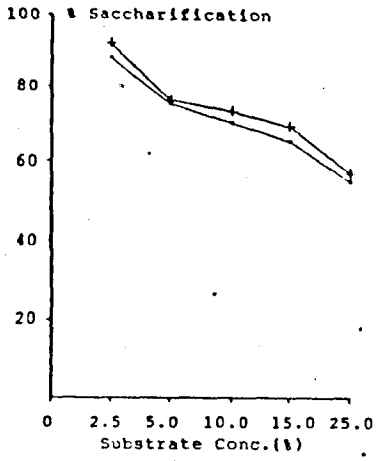

Fig. 5 C: Effect of Subsrate conc. on saccharification L: 50°C, 4.5 pH, 40 FPU Cellulose, 48 h. A. leptopus (•---•), Sugarcane (+--+)

$$\% \text{ Saccharification} = \frac{\text{Reducing sugars} \times 0.9}{\text{Total carbohydrates in substrate}} \times 100$$

The saccharification of cellulose should yield a high sugar content per enzyme unit. Optimisation of process parameters plays an important role in the economy of saccharification process. To maximize yield, basic variables such as pretreatment, temperature, pH and enzyme and substrate concentrations were optimised.

Purified cellulose showed no loss of activity, when stored below 4° for one year. Alkali cooking and alkaline H_2O_2 treatment showed 4 and 5-fold increase in yield over untreated substrate, respectively (Fig. 1). Although mechanism of lignin degradation is largely unknown, it is thought that oxidants like H_2O_2 may play an important role[12]. Gould and Freer (1984)[10] reported 50% more lignin removal with alkaline H_2O_2 treated wheat straw, corn stalks and husks. Presently the conversion was completed in 24 h. Extension of reaction time to 48 h and further had a very minimal effect on saccharification (Fig. 1).

For convenience, we compared the present results with the results obtained with sugarcane (**Saccharum officinarum**) leaves by treating them in same manner as **A. leptopus** leaves. 50°, pH 4.5, cellulose 120 FPU/g substrate, substrate 2.5% were found to be optimum (Fig. 2-5). Data from Fig. 4 indicates that 3 times increase in enzyme content (40-120 FPU) increase the yield by 10% and 13% only, with **A. leptopus** and sugarcane, respectively. Hence, 40 FPU per gram substrate was selected as ideal. Increase in substrate quantity (5-25%) decreased yield due to difficulties in stirring and end-product inhibition. Analysis at 24 h and 48 h was given in Table 1. It is apparent that **A. leptopus** conversion was over in a short time (24 h), compared with other substrates (48 h).

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REFERENCES

1. Ghose T K and Ghosh P, **J. Appl. Chem. Biotechnol.**, 1978, 28, 309.
2. Martin C, Negro M.J, Alfonsel M, and Saez R., **Biotechnol. Bioeng.**, 1988, 32, 341.
3. De Padilla F H and Hoskins F. H, **J. Agri. Food Chem.**, 1968, 16, 735.
4. Updegraff D. M, **Biotechnol. Bioeng.**, 1971, 13, 77.
5. Ooshima H, Ishitani Y and Harano Y, **Biotechnol, Bioeng.**, 1985, 27, 389.
6. Ladisch M. R, Ladisch C M and Tsao G T, **Science.**, 1978, 201, 743.
7. Saddler J N, Brownell H. H, Clermont L. P and Levitin N, **Biotechnol. Bioeng.**, 1982, 24, 1389.
8. Ghose T. K, **Biotechnol. Bioeng.**, 1969, 11, 239.
9. Mandels M, Andreotti R and Roche C, **Biotechnol. Bioeng. Symp.**, 1976, 6, 21.
10. Gould J. M and Freer S.N, **Biotechnol. Bioeng.**, 1984, 26, 628.
11. Miller G. L, **Analyt. Chem.**, 1959, 31, 426.
12. Forney L. J, Reddy C. A, Tien M and Aust S. J, **J. Biol. Chem.**, 1982, 257, 11455.

Effect of Isoxsuprine Hydrochloride on Onset of Labour in Pregnant Rats.

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The effects on spontaneous labour of Isoxsuprine hydrochloride (I), a β2-adrenoceptor agonist[1] were studied on pregnant rats. Oral administration of (I) from day 13 to 21 of gestation in dose of 2.5 mg/kg/day or higher effectively delayed the onset of labour, slight increase in duration of parturition (DOP) and average weight of pup (AWP) were observed. No adverse effects on the gestating animals or fetuses were noted. The rat model appears to be a simple, reliable and cost- effective method for evaluating uterine relaxants.

IN our search for alternative animal models for evaluating uterine relaxants we chose the rat model as it appears to have number of advantages over the existing test systems[2,3]. The period of second and third trimester of pregnancy when the incidence of premature labour is significantly higher[4] was chosen for the present study. Instead of measuring uterine tone, the onset of parturition was noted to record the delay in labour. Other related parameters such as duration of parturition (DOP), average litter size (ALS), average weight of pup (AWP), the mortality of mother and fetus and bleeding at parturition were also noted.

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