

Short Communication

Saccharification of banana agro-waste by cellulolytic enzymes

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Banana is major cash crop of this region generating vast agricultural waste after harvest. The agro-waste including dried leaves and pseudostem after harvest was used as substrate for the release of sugars. Saccharification of banana agro waste by cellulases of *Trichoderma lignorum* was investigated. The steam treated agro-waste yielded 1.34 mg/ml of reducing sugars after 24 hr. The size of substrate affected saccharification where the smaller size (<120 μ) yield more sugars. Maximum sugars were released at pH 6.0 whereas 40°C was the optimum temperature. Thus, under these conditions the agro waste left behind for natural degradation can be utilized affectively to yield fermentable sugars which can be converted into other substances like alcohol.

Key words: Banana agrowaste, *Trichoderma lignorum*, cellulases, saccharification

INTRODUCTION

The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria. Cellulose is a potentially valuable resource for fibre, fuel and feed. Investigations into ability of microbes to degrade native and modified cellulose so far have revealed that only a few fungi possess ability to degrade native cellulose. A majority of microbes can however degrade modified cellulose. The cost of carbohydrate raw material influences the economy of many fermentation processes, hence the cost play a decisive role in future and scope of industries employing fermentation processes (Dale, 1987; Castellanos et al., 1995). A lot of emphasis had been given to screening of the agricultural wastes for release of sugars produced by hydrolysis of lignocellulosics. Thus, the reducing sugars released following the saccharification of available agricultural waste hydrolysis can be used for production of alcohol and other chemicals.

The saccharification of different agrowastes has been reported by other workers employing enzymes from different organisms. Vlasenko et al. (1993) investigated

30 potential cellulosic raw materials for saccharification by *Penicillium* cellulases. Farooq et al. (1994) studied the saccharification of Kallar grass straw with thermostable cellulases from several fungi including *Chaetomium thermophile*, *Trichoderma reseei*, *Sporotrichum thermophile*, *Aspergillus fumigatus*, *Torula thermophila* and *Humicola grisea*. Okeke and Obi (1995) reported the saccharification of agrowastes by cellulases and hemicellulases from two fungal isolates viz. *Sporotrichum pruinosum* and *Arthrographis* sp. While Castellanos et al. (1995) evaluated various hydrolysis conditions of skop (short fiber waste material from paper industry) and cellulosic material by cellulolytic enzymes of fungi.

In Maharashtra state, India, banana occupies an area of 46,900 hectare yielding 25, 29,300 tonnes of fruit with an average of 53.95 tonne/hectare. Nanded district of Maharashtra is one of the leading producer of banana (Anonymous, 2001). After the harvest of the fruits the whole plant (leaves, stem and rhizome) is left in the field for natural degradation, which takes several months. However, these wastes can be utilized for release of sugar.

The present paper reports the saccharification of banana agro waste abundant in the Nanded region and the condition affecting the saccharification of the banana agro waste such as time course, pH, temperature and particle size.

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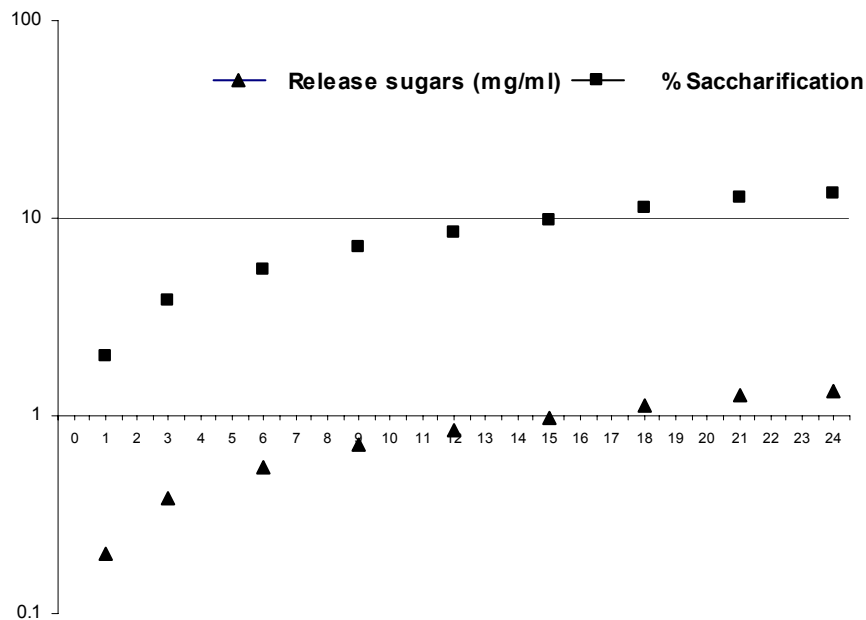


Figure 1. Time course for Saccharification of banana agro waste.

MATERIAL AND METHODS

Enzyme source

A wild strain of *Trichoderma lignorum* was isolated from the fields where banana was cultivated as ratoon crop continuously for seven years. *T. lignorum* was grown on the banana waste based medium for production of cellulolytic enzymes and assays were carried out as reported earlier (Baig et al., 2003).

The source of enzyme used in the study was the precipitated powder from the culture filtrate of the enzyme produced on banana agricultural waste medium containing 0.20 U/ml of FPase, 0.41 U/ml of CMCase and 0.24 U/ml β Glucosidase (Baig et al., 2003). The enzyme solution was prepared by dissolving the precipitated enzyme powders of *Trichoderma lignorum* in 50 mM acetate buffer pH 5.6.

Saccharification of substrates

The Banana agro waste (pseudostem, leaves, etc) used for saccharification were freshly collected from local farm following harvest. The waste was washed thoroughly with water and air dried. It was ground to powder using high speed pulverizer and sieved through different mesh sizes (US standard screen). In all the experiments, the size of particles was kept between 120 – 250 μ .

A suspension of substrate (10 g/l) was prepared in 50 mM acetate buffer (pH 5.0). This mixture was autoclaved at 15 lbs for 20 min for sterilization as well as heat treatment prior to release of sugar. Exactly 15 ml of each substrate suspension was taken in stoppered 100-ml Erlenmeyer flasks. The substrate was added to 5 ml of culture filtrate obtained from cellulolytic fungi under study. Saccharification was performed in a water bath shaker at $27 \pm 2^\circ\text{C}$ for 24 h. The resultant supernatants following centrifugation (2500 g, 15 min) were assayed for total reducing sugars using DNS method (Miller, 1959). The release of sugar is expressed as equivalent to glucose.

To determine the optimum temperature of saccharification, the reaction mixture was incubated at different temperature ranging from 20°C to 60°C . The optimum pH was determined by adjusting

the pH of the reaction mixture within the range of 3.5 to 7.5. The percentage saccharification was calculated as:

$$\text{Saccharification (\%)} = \frac{\text{glucose (mg/ml)} \times 100}{\text{Substrate (mg/ml)}}$$

RESULTS AND DISCUSSION

Enzymatic conversion of cellulose to food, fuel and chemical feedstock is a well-established process. However, high cost of cellulases production has hindered use of this enzyme in industry. The enzymatic conversion of the carbohydrate part of lignocellulosic material has received considerable interest during recent years. This source of raw material is available in abundance and generally free of cost. This could be converted into fermentable sugars. To reduce the production cost and enhance the formation of cellulases, which are both essential for the utilization of the carbohydrate components of lignocellulosics, different strategies can be applied.

Lignocellulosic waste of Banana plant left over otherwise for natural degradation in field was effectively used as component in the medium for the production of enzymes (Baig et al., 2003). Subsequently these enzymes produced on the medium containing banana agro waste can be further implicated in the saccharification of the same agro waste.

T. lignorum synthesized cellulases were used for saccharification of agro waste. The cellulolytic enzyme complex when incubated with the agro waste released sugars. The degree of saccharification was assayed on the basis of release of reducing group. The amount of reducing sugars increased with time of incubation in the

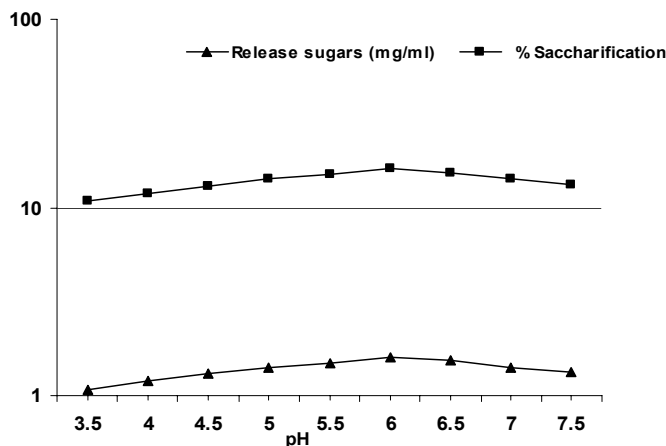


Figure 2. Effect of pH on saccharification of banana agro waste.

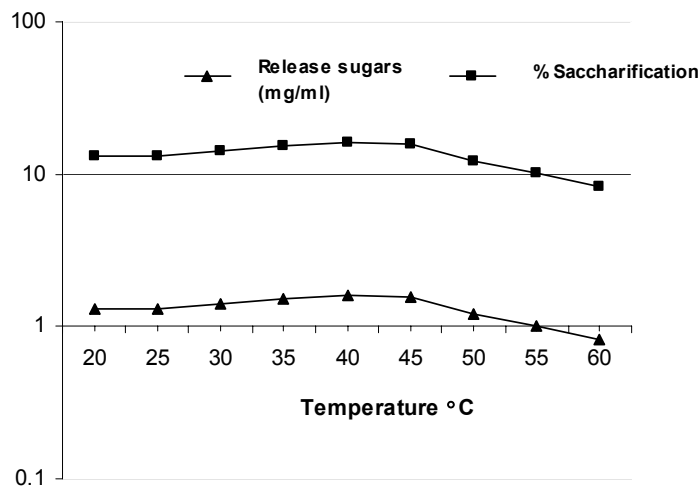


Figure 3. Effect of temperature on saccharification of banana agro waste.

presence of the enzyme. The maximum amount of reducing sugars was released at the end of 24 h (Figure 1). The resistance of agrowaste lignocellulosic material hydrolysis, so in all the experiment the pre-treated agro waste (with steam) was used.

Subsequently the effect of pH and temperature on the release of the reducing sugars was also studied. It is clear from the Figures 2 and 3 that the optimum pH for the release of maximum sugar was 6.0 whereas 45°C was optimum temperature. The optimum pH for saccharification was the optimum pH for synthesis of cellulolytic enzyme by fungus (Baig et al., 2003). Similarly the temperature for synthesis of enzymes was optimum for the saccharification of agro wastes in all cases to enzymatic hydrolysis can be attributed to lignin content of the material. Many researchers have studied the effect of agrowaste pre-treatment by alkali or steam (Okeke and Obi, 1994; Kirk and Farrel, 1987; Durand et al., 1984; Waldron and Eveleigh, 1986; Ekhlund et al., 1990). Pre-

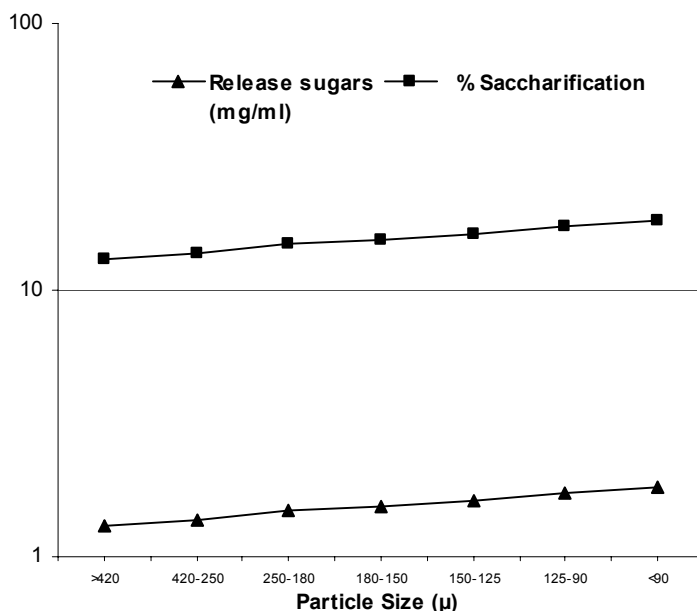


Figure 4. Effect of particle size on Saccharification of banana agro waste.

treatment of lignocellulosic material enhances enzymatic studied. However maximum saccharification was achieved within the range of 30-45°C coinciding with the characteristic of mesophiles. Though much studies has been made with thermophilic fungi and thermo stable enzymes for saccharification, fewer studies of enzymes from mesophilic fungi are available. Higher saccharification rate have also been obtained but lower enzyme stability was recorded with thermophiles (Okeke and Obi, 1994). The study indicated that maximum amount of reducing sugar can be obtained keeping the particle size the small (< 90, Figure 4).

The saccharification of banana waste by enzyme produced by *T. lignorum* indicates the enzymes specificity towards the substrates. Enzymes synthesised on banana agrowaste medium released more glucose. This can be attributed to the other enzymes like ligninase and hemicellulases in the agro-waste medium, synthesised along with cellulases.

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