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Simultaneous Saccharification and Fermentation of Waste Newspaper to Ethanol

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Abstract

Cellulase produced by Trichoderma reesei *QM 9414 exhibited higher filter paper enzyme (FPase) and* endo-*1,4-glucanase (Cx) activities when grown on 1% (w/v) microcrystalline cellulose powder than when grown on untreated or alkali-treated newspaper substrates. However, the cellulase produced on alkali-treated newspaper substrate exhibited a maximum degree of saccharification of alkali-treated newspaper during 72 h incubation compared to the cellulases obtained on other cellulosic substrates. The simultaneous one-step saccharification of alkali-treated newspaper using* T. reesei *cellulase and fermentation to ethanol by* Saccharomyces cerevisiae *gave increased ethanol with increased substrate up to 5% paper, the maximum used.*

Key words: Ethanol fermentation, cellulase, saccharification.

INTRODUCTION

The potential of utilizing global reserves of lignocellulosic materials for conversion to useful fermentation products such as fuel alcohol has generated extensive interest during the past few decades. Newspapers are published daily in every part of the world. After serving this purpose they become waste. Newspaper was chosen as a substrate for ethanol production because of its high cellulose content, consistent composition, low cost, ready availability and freedom from a high percentage of inorganic substances which might affect the fermentation kinetics adversely (Pamment *et al.*, 1979; Franzidis & Porteus, 1981).

A major step in the conversion of cellulose to ethanol or other useful chemicals is the breakdown of cellulose to glucose. Two methods are currently suggested as economically feasible: acid or enzymatic hydrolysis. Each method has its advantages and disadvantages, but the overriding factors in the long run must be low energy requirement and low pollution. Enzymatic hydrolysis achieves both of these ends as it is not only energy-sparing but also avoids the use of toxic and corrosive chemicals.

The present study was aimed at finding the potential of bioconversion of waste newspaper cellulose to ethanol by simultaneous saccharification by *Trichoderma reesei* cellulase and fermentation by *Saccharomyces cerevisiae*.

METHODS

Organisms

Trichoderma reesei QM 9414 used for cellulase production and *Saccharomyces cerevisiae* NRRL 2358/Y132 used for ethanol production were obtained from the departmental culture collection. The mould culture was maintained on PDA and yeast culture maintained on malt extract–glucose–yeast extract–peptone (MGYP) agar; both were stored at 4 ± 1°C.

Analyses

The method of Sternberg (1976) was followed for the production of cellulase using the medium of Mandels *et al.* (1974) modified by Warzywoda *et al.* (1983). The procedure of Andren *et al.* (1976) was followed for the saccharification of newspaper using a cellulase preparation from *T. reesei*

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and was calculated as:

% Saccharification

$$= \frac{\text{reducing sugar (mg/ml)} \times 0{\cdot}9 \times 100}{\text{initial substrate (mg/ml)}}$$

The method of Ooshima *et al.* (1985) was followed for simultaneous saccharification and fermentation of newspaper. Moisture and ash contents of newspaper were estimated as described by Ward *et al.* (1978), and total organic matter using the method of Graham (1948). The method of Updegraff (1969) was followed for the determination of cellulose and lignin by the procedure of Johnson *et al.* (1961) modified by Deschamys *et al.* (1981). The method of Mandels *et al.* (1974) was followed for alkali treatment of newspaper for the purpose of delignification. The dinitrosalicylic acid method of Miller (1959) was followed for the estimation of filter paper enzyme (FPase) and *endo-β*-1, 4-glucanase (Cx) activities. The enzyme activity is expressed as μM glucose released per minute per millilitre of supernatant. A Neish (1952)-type distillation plant was used for the distillation of fermented liquid. Distilled samples were analysed using gas liquid chromatography.

RESULTS AND DISCUSSION

The Hindustan Times, an Indian daily, contained (%): cellulose 85, lignin 12, moisture 2·7, ash 2·3, total organic matter 97·5 and total organic carbon 56·6. Franzidis and Porteus (1981) also reported 85–90% cellulose and 0–15% lignin in paper. The treatment of newspaper with 2% alkali resulted in 72·2% dry matter recovery and loss of 52·4% lignin content.

The cellulase production by *T. reesei* on crystalline cellulose and newspaper substrates (1% w/v) during 7 days fermentation was FPase 1·4 and 1·1 units and Cx 148·3 and 26·1 units, respectively. The results are in agreement with the findings of Mandels *et al.* (1974) who reported 0·9 units of FPase and 24 units of Cx activities from 1% Jay Bee newspaper. The comparison of FPase and Cx activities from alkali-treated and untreated newspaper substrates clearly showed a positive effect of alkali treatment.

Alkali treatment of the newspaper resulted in an increased degree of saccharification, from 9·2 to 50·4%, during 72 h incubation using cellulase produced on cellulose powder. Poor saccharification values were noted with the cellulase produced on untreated newspaper substrate. However, when cellulase obtained by growing *T. reesei* on alkali-treated newspaper was used, the saccharification of alkali-treated newspaper substrate was 55·8% during 72 h incubation. In general, the extent of saccharification was greater with alkali-treated than with untreated newspaper irrespective of the substrate used for cellulase production by *T. reesei* (Fig. 1). The results are in accordance with the findings of Chavadej *et al.* (1980) and Furusaki *et al.* (1986) who reported

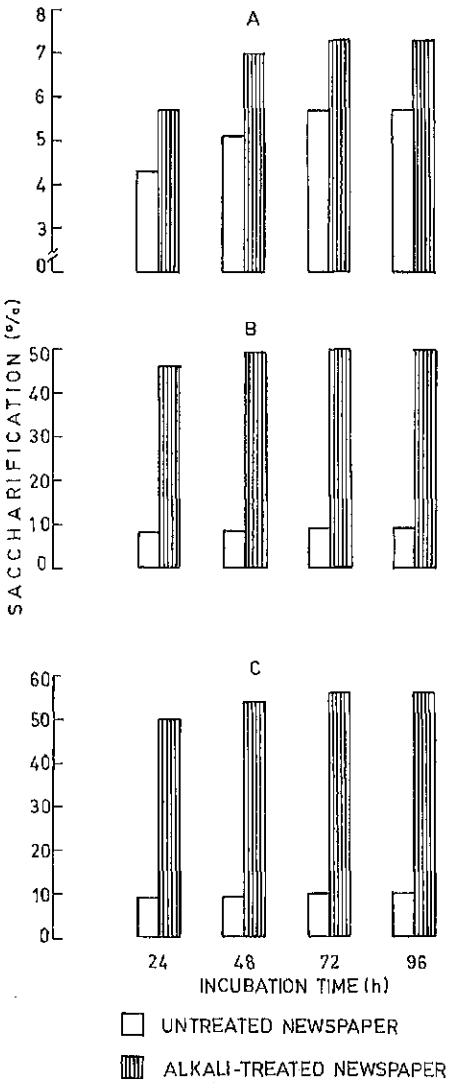

Fig. 1. Saccharification of newspaper by *Trichoderma reesei* QM 9414 cellulase obtained from (A) untreated newspaper, (B) cellulose powder and (C) alkali-treated newspaper: 5 g paper, 10 ml citrate buffer (0·5 M, pH 4·8), 50 ml *T. reesei* QM 9414 culture filtrate, water to 100 ml. Shaken incubation at 50°C.