Enzyme Production
of *Trichoderma reesei* Rut C-30
on Various Lignocellulosic Substrates

CHUL SEUNG SHIN, JOON PYO LEE,
JIN SUK LEE, AND SOON CHUL PARK*

Biomass Research Team, New and Renewable Energy Department,
Korea Institute of Energy Research, 71-2 Jangdong Yusonggu,
Taejon, Korea, E-mail: bmscpark@kier.re.kr

Abstract

Economical production of cellulase enzyme is key for feasible bioethanol production from lignocellulosics using an enzyme-based process. On-site cellulase production can be more feasible with the process of separate hydrolysis and fermentation (SHF) than with simultaneous saccharification and fermentation, since the cost of enzyme is more important and a variety of substrates are available for the SHF process. Cellulase production using various biomass substrates available for SHF, including paper sludge, pre-treated wood (steam exploded), and their hydrolysis residues, was investigated in shake flasks and a fermenter for their productivities and titers. Among the newspaper sludge, office paper sludge, and steam-exploded woods treated in various ways, the steam-exploded wood showed the best properties for substrate in cellulase production. The best titer of 4.29 IU/mL was obtained using exploded wood of 2% (w/v) slurry in the shake flask, and the titer with the same substrate was duplicated to about 4.30 IU/mL in a 3.7-L fermenter. Also, the yield of enzyme reached 215 IU/g of substrate or 363 IU/g of cellulose. Despite various pretreatment attempts, newspaper and office paper substrate was inferior to the exploded-wood substrate for cellulase production. However, hydrolysis residues of papers showed quite promising results. The hydrolysis residue of office paper produced 2.48 IU/mL of cellulase in 7 d. Hence, the utilization of hydrolysis residues for cellulase production will be further investigated in the future.

Index Entries: Cellulase production; lignocellulosic substrates; waste papers; steam-exploded wood.

*Author to whom all correspondence and reprint requests should be addressed.*
Introduction

Economic evaluation of a future full-scale plant for production of ethanol from various lignocellulosic materials, such as softwood, hardwood, waste paper, and wheat straw, is frequently based on enzymatic hydrolysis instead of conventional acid hydrolysis because of the higher ethanol yield and lower by-product formation (1). Moreover, modeling and sensitivity analysis of ethanol production process economics has shown the outstanding cost of enzyme production (2) and enzyme production facilities (3). Hence, economical enzyme production from proper substrates is key to developing enzyme-based bioethanol production from lignocellulosic biomass. Even though the commercial cellulase complex is normally utilized in the test run of a pilot plant for ethanol production, until now, the utilization of lignocellulosic substrate for the on-site production of cellulase enzyme and ethanol could not provide a streamlined and economic ethanol production process with reduced production cost (4). Cellulase production from pure cellulose, or from soluble sugars such as lactose, cellobiose, and sophorose, was studied thoroughly in submerged culture by a number of investigators. However, the production of the enzyme with those high-value substrates was not economically feasible for the large-scale ethanol production process. Hence, wheat straw (5), bagasse (6), aspen wood (7), willow (8), yellow poplar (4), and waste newspaper (9-11) treated by physicochemical methods such as alkali treatment, steam explosion, pulverizing, or partial hydrolyzation were utilized as substrates to produce effectively the cellulase complex. Also, sulfite liquor (12) and corn steep liquor (13) were added to those substrates to induce and improve the productivity of the enzyme.

The aims of this study were to determine not only the substrates available for the economical production of cellulase complex but also the methods of the pretreatment of each substrate including waste newspaper, office paper, and oak wood. The paper wastes were treated by ammonia with hydrogen peroxide catalysts. On the other hand, the oak wood chip was steam exploded after dilute acid percolation or without percolation. In addition, the residue of the hydrolysis was examined as substrate for the production of the enzyme.

Materials and Methods

Substrates and Their Preparation

Waste Newspaper and Office Paper

Waste newspaper pulverized with a Wiley mill (Thomas Scientific model 3383, -40 mesh) and ball mill, disintegrated by a pulper (helical blade type, Korea), was utilized for the production of cellulase. The newspaper disintegrated by the pulper was further treated with ammonium hydroxide (30% [w/v] NH₃) with and without hydrogen peroxide (H₂O₂)