Electronic Journal of Biotechnology 20 (2016) 14–19

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Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research Article

# Enzymatic hydrolysis and fermentation of ultradispersed wood particles after ultrasonic pretreatment

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ARTICLE INFO

*Article history:*
Received 24 July 2015
Accepted 5 November 2015
Available online 12 February 2016

*Keywords:*
Bioethanol
Ligsnocelluloses
Yeast *Saccharomyces cerevisiae*

ABSTRACT

*Background:* A study of the correlation between the particle size of lignocellulosic substrates and ultrasound pretreatment on the efficiency of further enzymatic hydrolysis and fermentation to ethanol.
*Results:* The maximum concentrations of glucose and, to a lesser extent, di- and trisaccharides were obtained in a series of experiments with 48-h enzymatic hydrolysis of pine raw materials ground at 380–400 rpm for 30 min. The highest glucose yield was observed at the end of the hydrolysis with a cellulase dosage of 10 mg of protein (204 ± 21 units CMCase per g of sawdust).
The greatest enzymatic hydrolysis efficiency was observed in a sample that combined two-stage grinding at 400 rpm with ultrasonic treatment for 5–10 min at a power of 10 W per kg of sawdust. The glucose yield in this case (35.5 g glucose $l^{-1}$) increased twofold compared to ground substrate without further preparation.
*Conclusions:* Using a mechanical two-stage grinding of lignocellulosic raw materials with ultrasonication increases the efficiency of subsequent enzymatic hydrolysis and fermentation.



## 1. Introduction

Modern economic development is closely associated with bioenergy as fossil fuel resources are depleted, leading to constant increases in fossil fuel prices. One way to solve this problem is to produce biofuels from renewable raw materials [1,2]. Bioethanol is traditionally produced from substrates containing both starch and lignocellulose [3,4,5]. While technologies based on starchy raw materials are well-developed, implemented and widely used, commercial cellulosic ethanol technology is still in its infancy. The conversion of lignocellulosic biomass to ethanol involves three processes: (1) a pretreatment process to increase the digestibility of cellulose and hemicellulose in the feedstock; (2) an enzymatic hydrolysis process to recover fermentable sugars from the pretreated material; and (3) a fermentation process to convert the obtained sugars into ethanol [6]. The main reason for this slow adoption is the recalcitrance of cellulose associated with lignin in the wood. Pretreatment technologies are aimed to increase enzyme accessibility to biomass and yields of fermentable sugars. Each pretreatment has a specific effect on the cellulose, hemicellulose and lignin fraction thus; different pretreatment methods and conditions should be chosen according to the process configuration selected for the subsequent hydrolysis and fermentation steps. In general, pretreatment methods fall into four different categories including physical, chemical, physico-chemical, and biological [4,7,8]. The main routes to produce ethanol from cellulose involve enzymes.

A major obstacle to complete substrate hydrolysis is the low availability of cellulose resulting from “shielding” by lignin [9,10]. Therefore, new methods and approaches are necessary to increase the availability of cellulose for enzymatic action. One approach is to break wood into ultrafine particles [11]. These technologies are limited by the need to increase the accessibility of fibers to enzymatic action. This limitation can be solved by mechanical treatment of raw materials to produce micron-sized particles [11,12,13] via dry grinding with mills of various designs (including ball mill). Other novel types of pretreatment such as microwaves, gamma radiation, and ultrasonication have been considered [14,15].

The aim of this study was to investigate the method of lignocellulose conversion in ethanol by enzymatic hydrolysis and subsequent fermentation with preliminary ultrasonic pretreatment.

## 2. Experimental

### 2.1. Materials

The object of this study was deresined *Pinus sylvestris* wood (Scots pine) with bark impurities no more than 5%, initial moisture content of 10.32% ± 0.37%, and no mechanical impurities. The corresponding air-dried wood was also studied. During the processing of lignocellulosic raw materials, knots and large splints were removed

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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2015.11.007

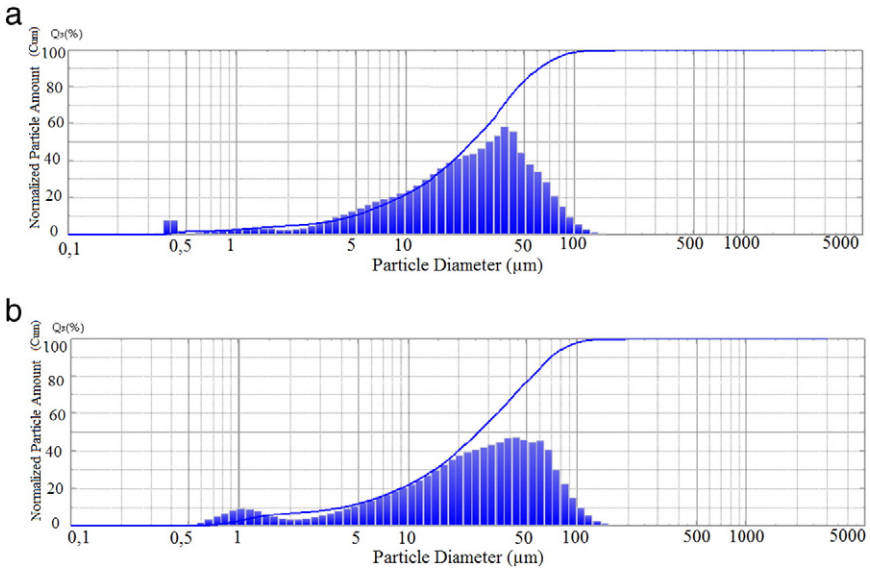


**Fig. 1.** Size dispersion of particles obtained by grinding of pine wood on a ball mill PM400 at 380 rpm for 30 min: (a) without treatment (initial moisture 18%), (b) after drying (initial moisture 10%).

by excluding particles with sizes greater than 10 ∗ 2 mm. The wet raw material was dried via convection heating in ventilated drying ovens at 50°C until 10% of the initial moisture remained.

### 2.2. Raw materials grinding

Grinding was performed using a one- or two-stage scheme. In the one-stage, raw material destruction is performed on a knife mill LZM-1 M at 15,000 rpm for 5 min. In the two-stage, first raw material destruction is performed on a knife mill LZM-1 M at 15,000 rpm for 5 min and then on vario-planetary ball mill PM400 set at 380–400 rpm for 20–30 min. Each loading of chips was no more than 70 g in mass. This loading corresponded to 45–55% of the grinding chamber volume, accounting for the volume of the grinding elements. The grinding period consisted of 2 min of operation followed by 2 min of cooling and 1 min of operation followed by 2 min of cooling.

### 2.3. Raw material pretreatment

For ultradispersed chip particles, ultrasonication pretreatment was performed using a UZG 2–4 M unit (ultrasonic generator, manufactured in Russia) with an output power of up to 6 kW and a resonance frequency of 16.8–9.2/20.5–23.5 kHz. Each treatment lasted up to 25 min. As a control, we used ultradispersed chips that were not pretreated.

### 2.4. Enzyme hydrolysis

For enzyme hydrolysis, highly active preparations (EP) were obtained from recombinant strains of *Penicillium verruculosum* 221–151. Preparation contained 834 ± 33 mg protein g. Enzyme activities in the preparation were: CMCase — 14.9 ± 0.6 U/mg, β-glucanase — 19.9 ± 0.8 U/mg, against MCC — 0.54 ± 0.02 U/mg, xylanase — 15.0 ± 0.6 U/mg, and cellobiase — 0.73 ± 0.03 U/mg, against n-NPG — 1.25 ± 0.05 U/mg. Preparations were also obtained from *P. verruculosum* F10. Preparation contained 738 ± 30 mg protein g. Preparation had activities of CMCase — 4.9 ± 0.2 U/mg, β-glucanase — 10.4 ± 0.4 U/mg, against MCC — 0.2 ± 0.01 U/mg, xylanase — 3.2 ± 0.2 U/mg, cellobiase — 111.4 ± 5 U/mg, against n-NPG — 55.3 ± 2.2 U/mg. The following EP dosages were used: cellulase — 2, 5 or 10 mg of protein per g of substrate, cellobiase — 5 mg of protein per g of substrate (or for cellulases — up

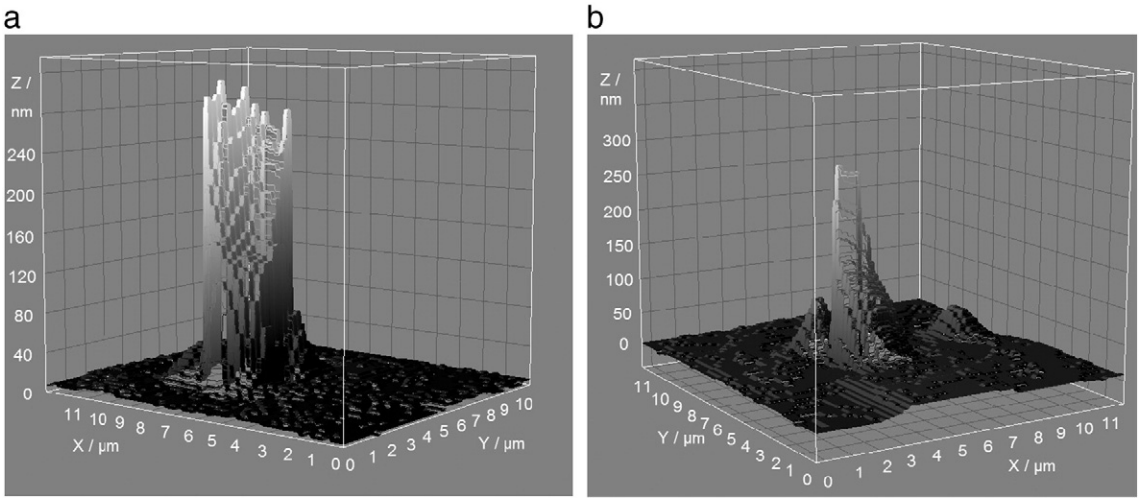


**Fig. 2.** 3-dimensional models of pine wood UDP obtained by grinding on PM400 for 30 min at 380 rpm: (a) without preliminary drying (initial moisture 18%); (b) after thermal drying at 75°C for 6 h (initial moisture 10%).