

# Effect of agitation speed on enzymatic saccharification of dry-pulverized lignocellulosic biomass



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## ABSTRACT

A vibration mill using cog-ring media, called a “tandem-ring mill”, was developed to achieve high-impact pulverization of lignocellulosic biomass for producing bio-ethanol. Instead of the ball medium in a conventional vibration mill, it has a cog-ring medium. Japanese cedar powder pulverized by the tandem-ring mill in a dry condition was pulverized to 20 μm particle diameter and 13% crystallinity index. Saccharification efficiency in enzymatic saccharification of Japanese cedar powder of greater than 70% was reached based on holocellulose. The Japanese cedar powder pulverized using the tandem-ring mill is suitable for bioethanol production. For this study, the Japanese cedar powder was prepared using a two-batch-type tandem ring mill in 60 min pulverization. Then, the agitation speed effect on Japanese cedar powder pulverization for enzymatic saccharification was investigated. Results show that the enzymatic saccharification efficiency of pulverized Japanese cedar powder increased continuously with decreasing agitation speed until 30 rpm. The best yields of 77% at 1 L and 75% at 3 L were obtained at 30 rpm agitation. The pulverized Japanese cedar powder produced by the tandem-ring mill was useful for enzymatic saccharification. Moreover, low agitation speed for saccharification of Japanese cedar powder pulverized by the tandem-ring mill might provide high saccharification efficiency.

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## 1. Introduction

Development of a bio-ethanol production technology, comprising pretreatment, enzymatic saccharification, and microbial fermentation of lignocellulosic biomass, has become an unavoidable trend to produce energy resources in many countries. Bio-ethanol from lignocellulosic biomass is expected to realize a sustainable fuel and to decrease greenhouse gas emissions. Lignocellulosic biomass is fundamentally composed from cellulose, hemicellulose, and lignin. Both cellulose and hemicellulose are collectively designated as holocellulose. Enzymes are well known to hydrolyze holocellulose effectively to produce monosaccharides such as glucose, arabinose, and xylose. Furthermore, microbial fermentation of these monosaccharides produces bio-ethanol.

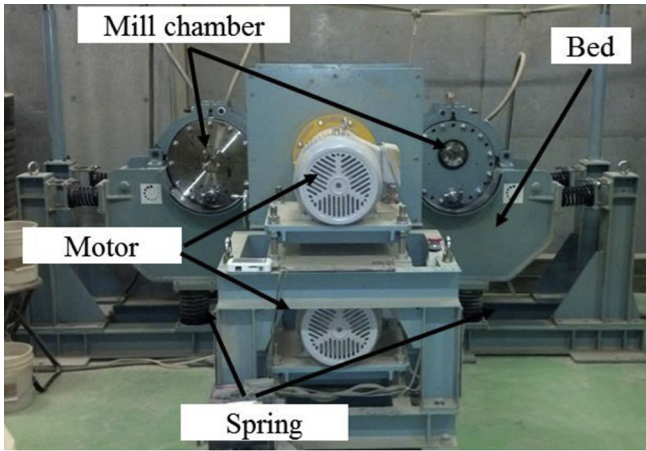
Many enzymatic hydrolysis effects on saccharification are still studied and discussed, especially the effects of biomass concentration [1,2], agitation speed [3], and enzyme dosage [4]. However, the enzymatic hydrolysis reaction of biomass is also affected by the state of biomass substance, particularly by the particle diameter

and crystallinity of cellulose. It is therefore important to develop a saccharification process corresponding to biomass pretreatment. Our earlier research clarified that the mean particle size of 20–30 μm and cellulose crystallinity index of 8–12% are the optimum conditions for high enzymatic saccharification using a normal vibration ball mill. The important factors in vibration milling were the compressive force and shearing force for particles in high-speed contact. Based on that knowledge, a vibration mill with cog-ring media, called a ‘tandem-ring mill’, which is a conventional vibration mill with the ball medium replaced by a cog-ring medium, was developed to achieve high-impact pulverization and to attain effective pretreatment of lignocellulosic biomass [5–7]. Japanese cedar powder pulverized using the tandem-ring mill showed high enzymatic saccharification efficiency greater than 80% in a tube test. On a commercial scale, it was necessary to develop an enzymatic saccharification agitation system corresponding to a large scale to achieve high saccharification efficiency in a short time. However, enzymatic hydrolysis effects on saccharification in an agitation system have remained unexplained.

This study investigated effects of agitation speed and enzyme loading on enzymatic saccharification using a pulverized Japanese cedar powder with the tandem ring mill using two scales of reactors, with 1 L and 3 L separable flasks, during 48 h processing.

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(a) Front view



(b) Bird's-eye view

Fig. 1. Appearance of two batch type tandem-ring mill.

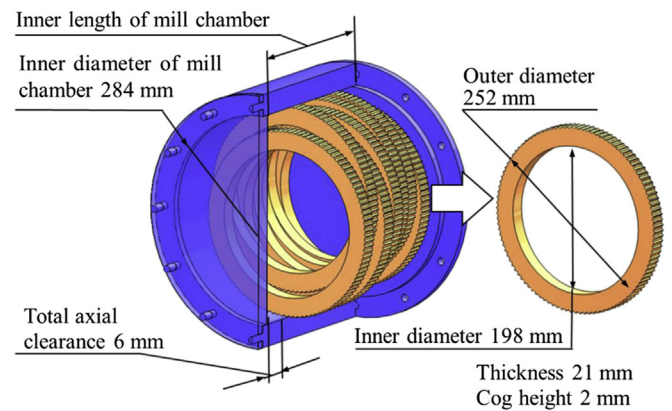
2. Tandem-ring mill

Fig. 1 presents a two-batch type tandem-ring mill that consists of a two-mill chamber, grinding media (tandem ring) inside the mill chamber, a vibrating band, two 3.7 kW motors, and eight support springs. The mill chamber was constructed with an outer case, a water jacket, and a 9-mm-thick steel liner. The mill chamber was shaken by rotary vibrations generated from the motor rotations combined with an unbalanced weight during pulverization examination. Additionally, the mill chamber was cooled by water through the water jacket during pulverization.

Fig. 2 shows the mill chamber interior and a schema of the tandem ring installed in the mill chamber. The tandem ring and biomass powder were enclosed in a mill chamber for pulverization. The mill chamber size had inner diameter of 284 mm and interior length of 216 mm. The tandem ring grinding media are annular with 2 mm high cogs on a 21-mm-thick steel circumference with an outer diameter of 252 mm and inner diameter of 198 mm. For the examination, a tandem ring, 10 media, and 800 g Japanese cedar powder were put into the mill chamber. Fig. 3 presents a schematic illustration of the basic motion of the tandem-ring mill for pulverization. The medium mass is denoted as  $m$ . The circular movement of the mill chamber moved the grinding media in a rolling motion with angular velocity  $\omega$  and revolution radius  $r$  along the chamber wall. This rolling motion of grinding media generated a centrifugal force  $F$  for pulverization, as given as Eq. (1).



(a) Interior of the mill chamber



(b) Schema of tandem ring media in mill chamber

Fig. 2. The tandem ring and biomass powder are enclosed in the mill chamber.

$$F = mr\omega^2 \tag{1}$$

The tandem ring rolling motion along the chamber wall provides a necessary pulverization force by both centrifugal compression force and the deformation force in tandem ring rolling motion.

3. Using the tandem-ring mill for biomass pulverization

Coarse Japanese cedar was prepared for tandem-ring mill pulverization. Coarse Japanese cedar conditions of initial diameter and moisture content were, respectively, less than 500  $\mu\text{m}$  and less than

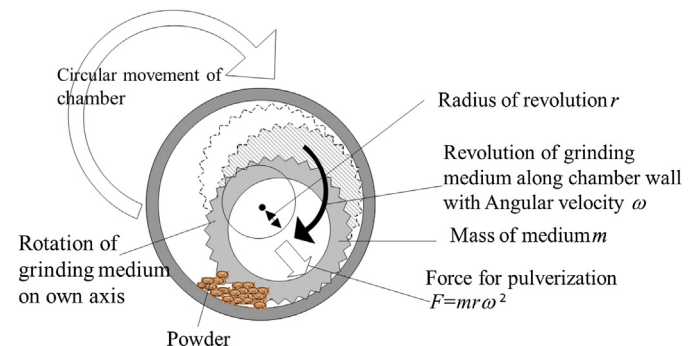


Fig. 3. Schematic illustration showing the basic motion of a tandem-ring mill.

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