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Bioconversion of waste office paper to gluconic acid in a turbine blade reactor by the filamentous fungus *Aspergillus niger*

Yuko Ikeda [a], Enock Y. Park [a,*], Naoyuki Okuda [b]

[a] *Laboratory of Biotechnology, Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan*
[b] *Technical Developments Department, Tsukishima Kikai Co., Ltd., 17-15, Tsukuda, 2-Chome, Chuo-ku, Tokyo 104-0051, Japan*

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Abstract

Gluconic acid production was investigated using an enzymatic hydrolysate of waste office automation paper in a culture of *Aspergillus niger*. In repeated batch cultures using flasks, saccharified solution medium (SM) did not show any inhibitory effects on gluconic acid production compared to glucose medium (GM). The average gluconic acid yields were 92% (SM) and 80% (GM). In repeated batch cultures using SM in a turbine blade reactor (TBR), the gluconic acid yields were 60% (SM) and 67% (GM) with 80–100 g/l of gluconic acid. When pure oxygen was supplied the production rate increased to four times higher than when supplying air. Remarkable differences in the morphology of *A. niger* and dry cell weight between SM and GM were observed. The difference in morphology may have caused a reduction of oxygen transfer, resulting in a decrease in gluconic acid production rate in SM.

Keywords: Recycle; Cellulase; Gluconic acid; Waste paper; Saccharification; *Aspergillus niger*

1. Introduction

Since the Industrial Revolution, people have benefited from petroleum resources. As a consequence, serious environmental problems have occurred such as air pollution, global warming and deforestation. Finding alternatives to petroleum products is thus an increasingly important objective for research. Recently, instead of using petroleum products, products utilizing biomass as raw materials have been developed for many markets. One of these, cellulolytic biomass, is known as a carbon neutral material because it does not increase the amount of carbon dioxide in the air.

Waste paper is one of the cellulolytic biomasses targeted to be recycled because it is a cause of environmental problems in Japan. Today in Japan about 30 million tons of paper is produced and consumed each year. The increased visibility of recycling has caused an increase of public awareness so that 66.1% of annual paper production is collected and 60.2% is reused (Terasawa, 2005). Although this recycling ratio is relatively high, it has not yet reached a satisfactory level. Excess paper that is not used by the Japanese market is exported to other countries. However, the price of paper has been rapidly decreasing and in this decade alone paper prices have been reduced by half. Moreover, when paper materials are recycled, they are usually turned into lower grade paper products; for example, conversions from office paper to magazine paper and from cardboard to sanitary products. With further recycling of paper, fiber length in the paper becomes shorter. Since the shortening of paper fibers decreases the quality of paper, the maximum ratio of paper-to-paper recycling is said to be

* Corresponding author. Tel./fax: +81 54 238 4887.
E-mail address: yspark@agr.shizuoka.ac.jp (E.Y. Park).

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65%. In addition, paper manufacturers are concerned about waste fibers which are not fit for recycling. This byproduct is disposed of by incineration or landfill without being reused. However, due to the shortage of new suitable disposal sites, environmental awareness, and the awareness of the greenhouse effect, these methods of disposal will be impossible in the near future. Finding alternative ways to recycle paper is an urgent necessity. One way of using waste paper is to decompose it to reducing sugars and to convert the sugars to value-added bioproducts; one such use of reducing sugars is fermentation into ethanol (Scott et al., 1994; Wayman et al., 1993).

Bioconversion of waste paper to L-(+)-lactic acid by the filamentous fungus *Rhizopus oryzae* has been studied previously by some of the present authors. Although the lactic acid yield from waste office paper hydrolysate was similar to that of a glucose medium, the production rate was inhibited by either xylose derived from hemicellulose or unknown compounds originating from paper pulp (Park et al., 2004).

This paper suggests an alternative way of using waste paper by converting it to gluconic acid. Gluconic acid has been used as a food additive, in sterilization solution or bleach in food manufacturing factories, and as salt in chemical components for medication. Using waste paper hydrolysate, the gluconic acid yield and production rate were compared to those obtained with a glucose medium in a flask and bioreactor using the filamentous fungus *Aspergillus niger*.

2. Methods

2.1. Enzyme hydrolysis

Waste office automation (OA) paper was used as the experimental material because of its availability. OA paper refers to the papers used in plain paper copiers (PPC), and waste OA paper is also defined as printed PPC paper after printing in a printer or copy machine. Four hundred grams of waste OA paper were cut into small pieces by a standard office shredder to rectangles 2 mm wide and 1.5 cm long, and were used for enzymatic hydrolysis without any further pretreatments (Park et al., 2001). They were dissociated into smaller pieces in 1500 ml of water using a blender for 1 min. Hydrolysis was done by an addition of 5% Acremonium cellulase AUSO 301 (1127 fpu/g, Meiji Seika, Co., Tokyo, Japan) by weight of the dried waste paper (Park et al., 2002). Hydrolysis was carried out at 45 °C for 2–3 days with sporadic mixing. During hydrolysis, pH was adjusted to 4–5 with 2 N HCl. After hydrolysis, the hydrolysate was separated from the slurry and immediately stored in a −35 °C freezer.

2.2. Microorganism and medium

The microorganism used in this study was *A. niger* IAM 2094, which consumes glucose as a carbon source and produces gluconic acid (Sakurai et al., 1989; Sankpal et al., 1999; Sankpal and Kulkarni, 2002). Spore formation was done on potato dextrose agar slants, and then the spores were stored in a −80 °C freezer.

The preculture medium was composed (g/l) of glucose, 50; yeast extract, 3; malt extract, 3; polypeptone, 5; calcium carbonate, 20. The calcium carbonate was used to adjust pH to 5–6. Production medium was composed (g/l) of carbon source, 50 or 100; $(NH_4)_2SO_4$, 1.35; Na_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.15; calcium carbonate, 60 (Sakurai et al., 1989). However, when enzymatic hydrolysate was used as a carbon source, glucose concentration was adjusted to 50 or 100 g/l with distilled water and then the ingredients of the production medium, except for glucose and $(NH_4)_2SO_4$, were added. The production medium containing enzymatic hydrolysate as a carbon source is defined as the saccharified solution medium (SM) and the production medium containing glucose as a carbon source as glucose medium (GM). All media and flasks were sterilized using an autoclave at 121 °C for 20 min.

2.3. Gluconic acid production

2×10^6 spores/ml of *A. niger* IAM 2094 were inoculated into 500 ml Erlenmeyer flasks containing 50 ml of preculture medium (10^8 spores/flask). The cultivation was carried out for 18 h at 30 °C at 120 strokes per min (spm) in a reciprocal shaker. For the gluconic acid production, 5 ml of preculture medium was inoculated into 500 ml Erlenmeyer flasks containing 50 ml of production medium. The cultivation was carried out at 30 °C at 120 spm in a reciprocal shaker for 72 h. To investigate the contribution of reducing sugars contained in the enzymatic hydrolysate to the gluconic acid production, xylose and cellobiose were used as sole carbon source. Each experiment was carried out in three different flasks; the average was taken as data. The cultivation was carried out at 30 °C at 120 spm in a reciprocal shaker.

The bioreactor, a turbine blade reactor (TBR) (2-1S, Sakura, Co., Ltd., Tokyo), was used for repeated batch culture of *A. niger* IAM 2094. The bioreactor was constructed of a glass cylinder with 800 ml working volume, where the culture space (400 ml) was separated from the agitation space (400 ml) by a stainless mesh cylinder and slit. Six hundred millilitres of production medium was put into the bioreactor before autoclaving. The medium in the agitation space flowed upward along the wall of the bioreactor, passed through the culture space, and returned to the agitation space through the central stainless mesh. The whole system was sterilized by autoclaving at 121 °C for 20 min. The inoculums size