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Use of Wastewater Sludge as a Raw Material for Production of L-Lactic Acid

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This study utilizes wastewater sludges to produce L-lactic acid, a precursor of biodegradable plastic. The high concentrations of cellulose contained in the sludge, derived from a paper manufacturing facility, have been found to be convertible to L-lactic acid at a rate as high as 6.91 g/L. To achieve such a high conversion rate, the sludge must be pretreated with cellulase. This pretreatment includes inoculation of the sludge with lactic acid bacteria, strain LA1, after the sludge has been subjected to enzymatic hydrolysis.

Introduction

Sludge is disposed in large quantities from wastewater treatment facilities and continues to be one of the most troublesome waste materials to deal with. The conversion of waste biomass into chemical feedstocks may reduce our dependence on petroleum-derived feedstocks while adding a value to hitherto value-less waste materials. The conversion of the cellulose in sludge to lactic acid is a step toward achieving environmental sustainability, while finding a new value in waste sludge. The present paper describes the hydrolysis of cellulose from sludge and the subsequent conversion to L-lactic acid, a precursor of biodegradable plastic.

A variety of biodegradable plastics have recently been developed (*1*). However, their high production costs are an important issue the industry must tackle to promote the use of biodegradable plastics. Thus, it is urgently needed to find some means to bring down the production costs. Peimin has estimated the production costs of L-lactic acid and has concluded that the cost of raw materials is ca. 40% of the total manufacturing costs (*2*). Therefore, achieving a low production cost for L-lactic acid is dependent primarily on what we choose for raw materials. If wastewater sludge can be efficiently used as a raw material for the production of polylactic acid, then a considerable reduction in costs would be possible.

How to best utilize the cellulosic materials has been a topic of active current research. Many studies have been published (e.g., refs *3*−*7*) regarding the production of ethanol, i.e., biofuel from cellulosic material. Some studies are concerned with production of lactic acid from cellulosic materials that is usable in various industries (*8*−*10*). Recently, a study has been done to use lactic acid, produced from waste paper, as a raw material for a biodegradable plastic (*11*). The present study is the first to produce lactic acid from wastewater sludge.

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Materials and Methods

Sludge and Lactic Acid Bacteria. The present study used two kinds of sludges derived from wastewater treatment facilities. One is from the fish-processing industry (sludge A) and the other from the paper-manufacturing industry (sludge B). These sludges differed not only in their moisture, organic matter, and elemental C, H, and N (Table 1) contents but also in their appearance when dried; i.e., sludge A appeared sandy while sludge B pulpy.

Lactic acid bacteria were isolated from sludge A. It is well-known that lactic acid bacteria usually require various kinds of micronutrient for growth factors; however, it is expected that the lactic acid bacteria isolated from sludge would grow and produce lactic acid in the sludge without the need for additional growth factors, because the bacteria is living in the sludge in the first place. We selected bacteria that produced a clear zone on an agar plate cultured at 32 °C for 7 days using the medium for lactic acid bacteria (yeast extract, 5 g; peptone, 5 g; glucose, 20 g; KH_2PO_4 2 g; distilled water, 1 L; pH = 6.1) supplemented with $CaCO_3$ (*12*). Acid-forming bacteria can be distinguished from others on the agar plate, and it is easier to identify the lactic acid bacteria from bacteria consisting only of acid-forming bacteria than from that with all the bacteria indigenous to the sludge. After cultivation at 32 °C for 7 days, colonies were successively streaked and purified and then stored on the slants of GYP medium (glucose, 10 g; peptone, 5 g; yeast extract, 10 g; $CH_3COONa\cdot 3H_2O$, 2 g; $MgSO_4\cdot 7H_2O$, 200 mg; $MnSO_4\cdot 4H_2O$, 10 mg; $FeSO_4\cdot 7H_2O$, 10 mg; NaCl, 10 mg; Tween 80, 500 mg; distilled water 1 L; pH = 6.8) at 4 °C. The lactic acid production of each acid-forming bacterium was then examined in the liquid medium by determining the concentration of L-lactic acid with the assay system, TC L-lactic acid (Boehringer Mannheim, GmbH), that utilizes an enzymatic reaction of NAD-linked L-lactic dehydrogenase with L-lactic acid for producing NADH. The concentration of L-lactic acid was determined by measuring the absorbance of NADH at 340 nm. The bacterium that most effectively produced L-lactic acid, strain LA1, was used for conducting further experiments. The strain LA1 grew at even a high concentration of glucose, 180 g/L, and the productivity of L-lactic acid at the early stage of the fermentation was 0.24 g/L/h, which is similar to the rates of production reported previously (*10*, *11*, *13*, *14*).

The LA1 strains was inoculated into sludge A and sludge B without pretreatment, and the production of L-lactic acid in each sludge was examined.

Analysis of Glucose and Polysaccharide Concentration in the Sludge. The sludges were dried at 105 °C for 2 days and then powdered by a mincer (<0.2 mm in diameter). The concentrations of glucose and polysacchardes that could be converted to glucose by hydrolysis were measured for each sludge. Glucose was extracted from the sludge by boiling it for 2.5 h in distilled water. The ratio of sludge to distilled water was 1:9 on a weight basis. In the analysis of the polysaccharide concentrations, the polysaccharides were hydrated by the method of Inoko et al., a method that was developed to measure the cellulose and hemicellulose concentrations in compost (*15*). Hydrolysis was carried out by adding 5 mL of an 80% H_2SO_4 solution to 1.25 g of dried sludge at temperatures 12−15 °C for 2.5 h. The mixture was then diluted with 175 mL of distilled water and boiled for 5 h.

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After the extraction of glucose or the hydrolysis of the polysaccharides, the concentration of glucose was determined by the Glucose C-II test WAKO (Wako Pure Chemical Industry, Ltd.) assay system that utilizes an enzymatic reaction of mutarotase and glucose oxidase with glucose for producing H_2O_2. The H_2O_2 then reacts with 4-aminoantipyrine and phenol by the action of peroxidase, producing red quinone pigment. The concentration of glucose was determined by measuring the absorbance of the pigment at 505 nm.

To confirm the validity of the measurement methods employed in determining the concentrations of glucose and polysaccharides, we added known quantities of standard substances, glucose and cellulose, to the dried sludge and then measured the concentrations again. It was confirmed that the concentrations of glucose and polysaccharides can be accurately determined by the methods adopted in this experiment.

Enzymatic Hydrolysis of the Sludge. Enzymatic hydrolysis of sludge B was carried out by mixing 30 g of dried sludge with a 500 mL acetate buffer solution (pH = 4.5) containing 0.1% cellulase and keeping it at 45 °C for 7 days. The cellulase used was Meicelase CEPB-5081 which was derived from *Trichoderma viride* and had an activity of 6180 U/mg (1000 U corresponds to an activity of 1 g of filter paper being completely degraded in 1 min) at 37 °C. It was ascertained that the cellulase exhibits its maximum activity at around 45 °C with pH = 4.5. The transient glucose concentration, with the progress of hydrolysis, was determined by the method described above. Aseptic conditions were maintained throughout the experiment.

Fermentation of Sludge Hydrolysate. In order to activate the microbial activity, the LA1 strain of lactic acid bacteria was precultured twice and successively (*16*): the LA1 strain was first inoculated into the centrifuging tube containing a 30 mL GYP liquid medium. After incubation of the culture at 30 °C for 2 days, the cell of LA1 was centrifuged at 15 000 rpm for 10 min, and the supernatant was exchanged for a 30 mL fresh liquid medium and then incubated again at 30 °C for 2 days.

After the second preculture, the cell of LA1 was centrifuged at 15 000 rpm for 10 min, and the supernatant was exchanged for 30 mL of enzymatic hydrolysate of sludge B, which was prepared as described above but incubated only 2 days and then autoclaved at 121 °C for 15 min to deactivate the cellulase. The cell density of the LA1 strain at the start of the hydrolysate fermentation was measured by dilution plating.

During the hydrolysate fermentation experiment, the concentrations of glucose and L-lactic acid were measured daily by the method described above. The cell density of the LA1 strain in the sample was also measured by the dilution plating method.

Results and Discussion

It was first confirmed that lactic acid was not produced when the LA1 strain was inoculated into the two sludges without pretreatment. The concentrations of glucose and polysaccharides that could be converted to glucose by acid hydrolysis were then determined (Table 1). No glucose was detected in either of the two sludges, whereas the quantity of polysaccharides that could be converted to glucose by acid hydrolysis was greater in sludge B than in sludge A. The polysaccharide concentration expressed as a glucose-equivalent concentration in sludge B was as high as ca. 33% (Table 1). This high concentration of polysaccharides in sludge B appears to be unique for a wastewater sludge. We have not yet analyzed the constituent polysaccharides in the sludge B. We assume, however, that these polysaccharides include cellulose, since the sludge was obtained from a wastewater treatment facility of the paper manufacturing industry. As shown in Table 1, the sludge shows a low N content. In view of this, it may be assumed that a large quantity of cellulose flows out into the wastewater during the paper-manufacturing process, which then condenses into sludge, resulting in a high cellulose content in the sludge.

TABLE 1. Characteristics of Two Kinds of Sludges

	sludge A	sludge B
moisture content (%)	78.5	49.9
organic matter content[a] (%)	79.1	70.7
element content[a]		
carbon (%)	38.7	33.1
nitrogen (%)	9.30	0.51
hydrogen (%)	5.66	4.86
glucose (%)	0	0
polysaccharides[b] (%)	0.87	33.3

[a] Dry weight basis. [b] The concentration of polysaccharides was expressed in terms of glucose generated by hydrolysis (glucose equivalent concentration).

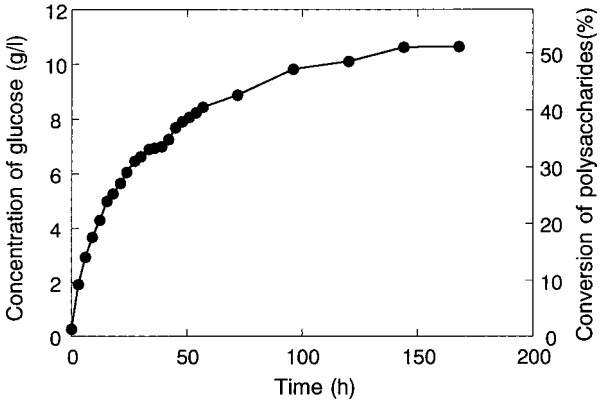

FIGURE 1. The course of glucose concentrations and the conversion of polysaccharides during the enzymatic hydrolysis of sludge B. The conversion of polysaccharides is defined as the ratio of the glucose concentration at a given time to the glucose concentration generated as a result of complete hydrolysis, i.e., acid hydrolysis.

Next, we performed an enzymatic hydrolysis of sludge B. The conversion of polysaccharides was assessed. Here, the conversion was defined as the ratio of the glucose concentration at a given time to the glucose concentration generated after acid hydrolysis has been completed. In the present experiment, 30 g of dried sludge (10 g glucose-equivalent polysaccharides) was hydrated in a 500 mL solution; therefore 20 g/L glucose was produced with complete hydrolysis of the polysaccharides. The final conversion of polysaccharides at 98 h is ca. 50%, which implies that 10 g/L of glucose is produced (Figure 1). The polysaccharide conversion did not exceed 50% probably because of the inhibition of cellulase due to glucose, i.e., through product inhibition, or the deactivation of cellulase during the hydrolysis, and/or the presence of polysaccharides other than cellulose in sludge B.

Finally, we attempted to produce L-lactic acid from the hydrolysate of sludge B. The cell density of the LA1 strain at the start of fermentation was 9.2 log10 CFU/mL. The final concentrations of L-lactic acid and glucose after 5 days of fermentation were 6.91 and 0.48 g/L, respectively, showing that L-lactic acid can be produced from the wastewater sludge by combining hydrolysis and fermentation processes (Figure 2). The LA1 strain produced L-lactic acid in the sludge hydrolysate without supplementation of any growth factors. The production yield of L-lactic acid from glucose was found to be ca. 0.9 g-lactic acid/g-glucose, which was quite high, indicating that a large portion of consumed glucose was

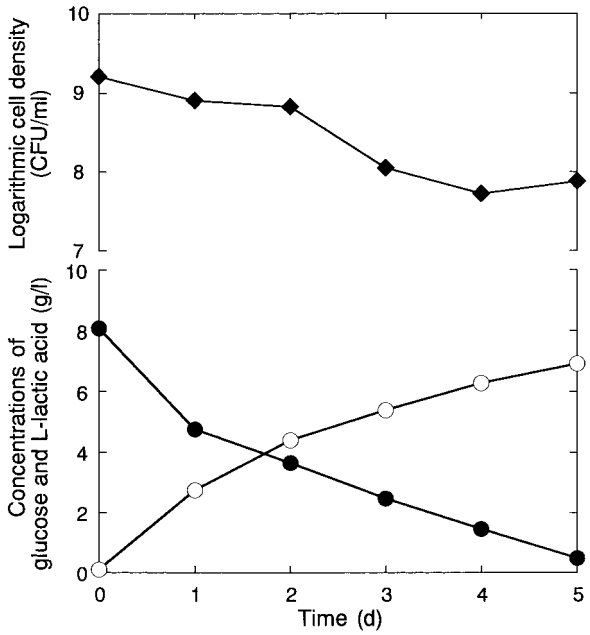

FIGURE 2. The course of concentrations of glucose and L-lactic acid, and of the cell density of strain LA1 during sludge hydrolysate fermentation (○: L-lactic acid, ●: glucose, ◆: LA1).

converted to L-lactic acid with high selectivity. The cell density gradually decreases as the fermentation progresses, finally reaching about 7.9 log10 CFU/mL (Figure 2). Hence it can be considered that the pH decrease associated with lactic acid production have inhibited the growth of the LA1 strain itself. In future experiments, we plan to control the pH during the fermentation of sludge hydrolysate by adding $CaCO_3$ or through the use of a pH stat instrument.

L-Lactic acid can be made from sludge B, which contains a high concentration of cellulose, provided that the hydrolysis and fermentation processes are combined. Sludge B is discharged at a rate of 35 000 t/year, which corresponds to ca. 1.4% of the total amount of sludge from the paper-manufacturing industry in Japan. By assuming that the polysaccharides contained in the sludge from the paper manufacturing industry can be completely hydrated into glucose and then completely converted to L-lactic acid, the production rate of polyactic acid amounts to 420 000 t/year, which corresponds to ca. 3% of the total plastic materials production rate in Japan.

Peimin has stated that it is most effective to reduce the cost of raw materials in order to minimize the total L-lactic acid manufacturing cost and that it may be possible to produce L-lactic acid with a production cost of less than $1 per kilogram from corn starch through the use of the newly developed air-lift bioreactor (*14*) in countries like the U.S.A. and China (*2*). The costs of using sludge as a raw material for the production of L-lactic acid have not yet been estimated. Extra costs will inevitably be incurred, when compared with the case of using corn starch as a raw material, for the hydrolysis of the cellulose in the sludge. On the other hand, corn starch must be acquired at a cost, while the sludge may be acquired free of charge. Furthermore the cost of sludge treatment could be charged on a revenue basis. It would be possible, therefore, to produce L-lactic acid from sludge at $1 per kilogram or less, although the cost may vary depending on the costs of hydrolysis and sludge acquisition. To reduce the overall costs for producing L-lactic acid from wastewater sludge, it is important to optimize the hydrolysis of the sludge as well as the fermentation process of the hydrolysate. Further study is needed to achieve a higher efficiency in hydrolyzing cellulose and attaining a higher concentration of L-lactic acid in the sludge hydrolysate.

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