Journal of Industrial Microbiology & Biotechnology (1997) 18, 10–14

Production of lactic acid from wastepaper as a cellulosic feedstock

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Lactic acid promises to be an important commodity chemical in the future as a monomer for the production of biodegradable polylactic acid (PLA). As the demand for lactic acid increases, the need to explore alternative feedstock sources and process options that are inexpensive and efficient is bound to gain importance. This paper reports the results of a study of the production of lactic acid from wastepaper as a representative cellulosic feedstock, using a batch, bench-scale simultaneous saccharification and fermentation (SSF) process. The effect on process performance of operating parameters such as pH, temperature, enzyme loading, solids concentration, and enzyme preparation has been examined. A lactic acid product yield of 84% of theoretical was achieved at a solids loading of 5%, using 25 filter paper units (FPU) of cellulase per gram of cellulose, at 45°C and pH 5.0. The pH and temperature of operation have been selected to achieve good performance of both the cellulase and the microoganism in the SSF process. Our studies show that a feedstock such as wastepaper offers considerable promise and opportunity in the future for development of a biomass-based process for lactic acid production.

Keywords: lactic acid; simultaneous saccharification and fermentation; biomass; cellulose; xylose; wastepaper

Introduction

Lactic acid is currently used in the food industry as an acidulent and preservative, and in the chemical industry for deliming, metal etching, and for cosmetic and textile applications. However, the most important application of lactic acid may yet be as a raw material for producing biodegradable and biocompatible polylactide products. These polymers have potentially large markets in commodity packaging, fabrication of prosthetic devices, and controlled delivery of drugs in humans. The successful development of these polymers and their penetration into existing markets could easily propel lactic acid into the commodity chemical category [8]. The substitution of existing synthetic polymers by biodegradable ones would also significantly alleviate waste disposal problems. Companies such as Archer-Daniels-Midland and Cargill have announced plans to manufacture lactic acid based on corn-derived glucose as a renewable feedstock [7].

Much work has been done on producing lactic acid from glucose-based feedstocks [3,5,12,18,19]. Product yields higher than 90% of theoretical have been reported in simple shake flask experiments and in bench-scale continuous setups. Process configurations that remove product during fermentation have been tested and have demonstrated enhanced volumetric productivity on the bench scale [10,19]. Studies performed on lactose fermentation also have demonstrated high product yields [10,11,17]. The efficient commercial production of lactic acid from simple sugars such as glucose will be important in meeting near-term cost targets of polylactic acid in the biodegradable polymer market.

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Received 9 January 1996; accepted 22 August 1996

Additional renewable feedstocks that offer an alternative are lignocellulosics. These waste products of industries such as corn fiber, corn stover, and wastepaper have a high content of polysaccharide (cellulose and/or hemicellulose). These can be converted to important fuel and chemical products provided cost-effective technologies exist for hydrolysis and fermentation. The use of these feedstocks will not only dispose of waste products that are posing a landfill problem, but will also convert them to value-added products. Converting these feedstocks requires the polysaccharides to be broken down into monomers and the sugars to be fermented to the final product.

Little systematic research has been done on the use of cellulosic waste feedstocks for producing lactic acid. Padukone *et al* [13] studied the achievable yields from pure cellulose in batch experiments. Venkatesh *et al* [16] suggested operating conditions for yield maximization through simulations of an empirical mathematical model. McCaskey *et al* [9] studied lactic acid production from acid-hydrolyzed municipal solid waste and reported a product yield of 65% of theoretical based on available carbohydrate.

In this article, we describe our systematic efforts to study wastepaper as a representative cellulosic waste feedstock for lactic acid production. We initially examined a range of available feedstocks to provide a rational basis for selecting wastepaper. The subsequent analysis of various operating conditions for achieving high yields from mixed paper should serve as a basis for more rigorous studies directed at process development.

Materials and methods

Microorganism

Lactobacillus delbrueckii B445 was obtained from NRRL, Peoria, IL, USA. Frozen working seed cultures were maintained at −70°C in 10% glycerol in FG medium [3].

Growth medium

The growth medium (designated as FG) contained, per liter: 0.6 g $MgSO_4 \cdot 7H_2O$, 11.3 g succinic acid, 7.25 g NaOH, 30 g yeast extract (Difco, Detroit, MI, USA), 1 g K_2HPO_4, 1 g KH_2PO_4, 0.03 g $MnSO_4 \cdot H_2O$, 0.03 g $FeSO_4 \cdot 7H_2O$. The pH was adjusted to 5 or 6 as required and the medium was autoclaved for 30 min at 121°C.

Feedstock

Sigmacell 50 (Sigma Chemicals, St Louis, MO, USA) was used as a control cellulosic feedstock. Wastepaper and corn stover were obtained from local sources and were tested without any chemical pretreatment. The cellulose content (% dry weight) of the feedstocks was: uncoated paper, 72.3; newspaper, 45.1; mixed paper, 60.5; and corn stover, 38.1. Mixed paper feedstock consisted of 25% newsprint, 15% magazines, 30% boxboard, and 30% white, uncoated sheet, all obtained from local sources. The loading of solids in an experiment depended upon the cellulose content of the feedstock.

Simultaneous saccharification and fermentation (SSF)

SSF studies were carried out in shake flasks and a bench-scale reactor. The reference conditions used were a substrate loading of 3% cellulose (approximately 5% solids), 25 filter paper units (FPU) of Laminex enzyme per gram of cellulose and FG as growth medium. Cellulase activity was measured as described by Ghose [4]. Shake flask experiments were carried out with 100 ml in 250-ml flasks (closed to the atmosphere by rubber stoppers) in an orbital shaker at 150 rpm. Samples were taken daily and pH adjustments were performed manually using 30% ammonium hydroxide (JT Baker, Phillipsburg, NJ, USA). Reactor studies were conducted in a New Brunswick Bioflow III model with a 1.5-L working volume in a 2.5-L vessel. Reactors were fitted with a pH probe, inoculation port, and a base addition port. A side port was used for sampling the slurry. The impeller shaft was fitted with a double set of modified Rushton blades, one set just below the surface of the slurry and the other close to the bottom of the reactor, to facilitate mixing. The pH was controlled automatically using 30% ammonium hydroxide.

Mixed paper was combined with medium in the reactor vessel and autoclaved for 45 min at 121°C. In order to keep the paper in suspension with enzyme and inoculum, initial mixing was done at 300 rpm. The agitator speed was reduced to 150 rpm after 1 h when the paper had been subjected to sufficient enzymatic action. Inoculum was prepared in two stages; this ensures that log phase cells at a high density are used to inoculate the experiment. Pre-inoculum was prepared from a frozen stock, and inoculum from the pre-inoculum; both were prepared in FG at pH 6 and 42°C. The SSF reactors were inoculated to a starting $OD_{590\ nm}$ of 1.0. The product yield was based on the theoretical yield of 1.0 g lactic acid g^{-1} cellulose [6]. The yield was corrected for dilution by base addition. The yield from experiments in FG were corrected for the product contribution from FG medium, that being 2.075 g of lactic acid produced per liter of FG components other than sugars. The productivity refers to volumetric productivity measured as grams of product per liter of reaction volume per hour.

Enzymes

All three enzymes examined are commercial preparations from *Trichoderma reesei* cultures. Laminex (Genencor International, San Francisco, CA, USA) had an activity of 64 FPU ml^{-1}. Genencor lot No. 17-92262-09, hereafter referred to as COG 1 (CPN, Martinez, CA, USA) had a cellulase activity of 98.7 FPU ml^{-1}, and EBT (Genencor) had an activity of 83.5 FPU ml^{-1}. We measured activity by filter paper assay as recommended by Ghose [4].

Analytical techniques

Samples were analyzed by HPLC using a Hewlett Packard Series II 1090 and a Biorad 87H organic acid column with UV detector, column temperature of 65°C and mobile phase of 0.01 N sulfuric acid. Injection volume was 10 μl. Lactate and glucose were also measured using a YSI 2700. The composition of the feedstock was analyzed by the Chemical Technologies Research Branch at NREL according to the method described by Ehrman and Himmel [2].

Results and discussion

Lactic acid production from selected cellulosic feedstocks

Corn stover and waste paper were selected for initial studies based on their abundance as waste feedstocks. Figure 1 shows the lactic acid yields produced from corn stover, various wastepaper feedstocks, and synthetic cellulose. Uncoated paper and mixed paper produced about 80% of theoretical product, which compared well with the yield obtained from the synthetic substrate, Sigmacell 50. The volumetric productivity of 1.0 g^{-1} L^{-1} h^{-1} with uncoated paper was threefold higher than that obtained with mixed paper. Both newspaper and corn stover showed considerably lower yields of 35–40% of theoretical. These results indicate a promise of uncoated and mixed paper for use, without pretreatment, as feedstocks for lactic acid production. Although prices of these feedstocks have varied considerably depending on the demand for recycled paper, mixed paper is cheaper than uncoated paper [14]. Therefore, mixed paper was selected as a representative feedstock for more detailed process analysis.

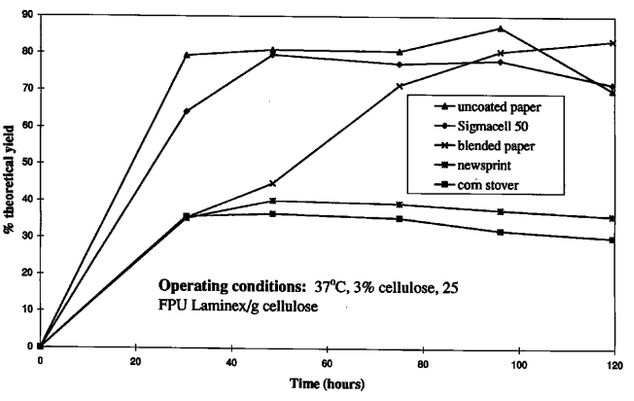

Figure 1 Lactic acid from lignocellulosic feedstocks.

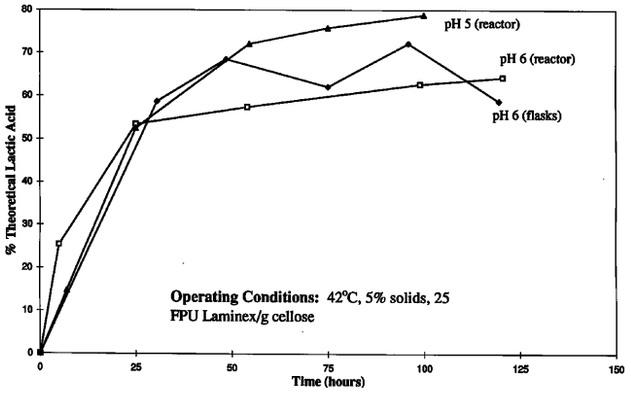

Figure 2 Effect of pH.

To develop a bench-scale process based on mixed paper as a feedstock, the effect of various operating parameters on performance was examined. The parameters studied were pH, temperature, enzyme loading, solids loading and nature of the cellulase preparation.

Effect of pH

Figure 2 shows the effect of pH on lactic acid yield. The studies using reactors indicated a product yield of 79% of theoretical at pH 5.0 compared to 65% of theoretical at pH 6.0. The optimum pH for Laminex activity is about 5.0 [15]. This effect of pH compares well with the model results presented by Venkatesh *et al* [16]. Interestingly, runs using shake flasks conducted at pH 6.0 produced a product yield of 72% of theoretical, higher than that observed in the reactor controlled at the same pH. This may be because the lower pH conditions maintained by the intermittent control in shake flasks improved the enzymatic action on cellulose. An operating pH of 5.0 was chosen for subsequent SSF reactor runs.

Effect of temperature

An increase in the operating temperature from 42°C to 45°C resulted in a yield increase from 79% to 84% of theoretical (Figure 3). The optimum temperature for Laminex activity is about 48°C [15]. The SSF process generally requires operating conditions which represent a compromise between the optimum conditions of the enzyme and the microorganism. The temperature of 45°C, which is closer to the optimum of Laminex than 42°C, brought about a significant enhancement in enzymatic activity without impairing the fermentation ability of the microorganism. Thus, this temperature represents a good compromise choice for SSF operation.

Effect of enzyme loading

Figure 4 shows product formation in shake flasks with varying enzyme loadings. An increase in enzyme loading from 7.0 to 25.0 FPU g^{-1} of cellulose increased the final yield of lactic acid from 56.9% to 72% of theoretical. However, the volumetric productivity measured at peak lactic acid levels was similar for all three enzyme loadings at 0.54 g L^{-1} h^{-1}. Two explanations may be offered for the decreased yield at the lower enzyme concentration: (i) deactivation of the enzyme after about 68 h; or (ii) inadequate contact in the solid-enzyme mixture for the lower loadings in the shake flasks. The constant volumetric productivity at all three loadings suggests the possible deactivation of the enzyme at the lower loadings. However, preliminary experiments indicate negligible enzyme deactivation during the SSF. Further experimentation needs to be carried out on enzyme-solid contacting to explain the results at the lower enzyme loadings and to devise strategies to achieve minimum enzyme usage.

Effect of solids concentration

A higher solids loading results in a higher process throughput, ie more solids processed per hour of operation. Hence, a greater productivity can potentially be achieved with high solids concentrations if yields are the same. The final level of lactic acid was about 31 g L^{-1} at the initial cellulose loading of 3%, representing 84% of theoretical yield (pH 5, 45°C). Figure 5 shows that the lactic acid yield at 8% solids (4.8% cellulose) was only 70% of theoretical.

The SSF at the higher loading was not limited by product inhibition because the selected strain of *L. delbrueckii* can tolerate up to 8% lactate (unpublished results, P Planes, S Schmidt, N Padukone, 1994). The low yield at the higher solids loading may be attributed to inadequate mixing as the higher solids concentrations present operational challenges. In the reactor runs, the 8% mixed paper formed a packed mass around the impeller shaft after sterilization. Therefore reactor contents subsequently were autoclaved in

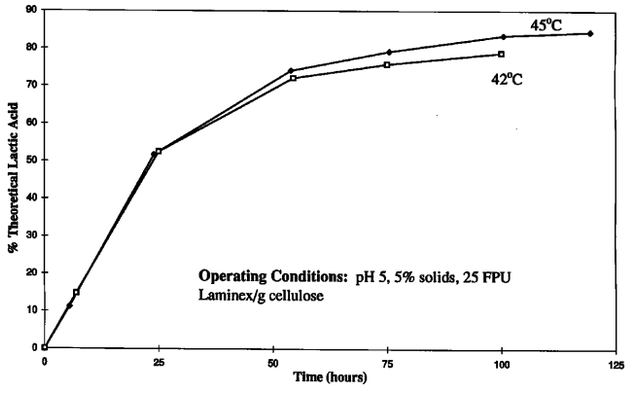

Figure 3 Effect of temperature.

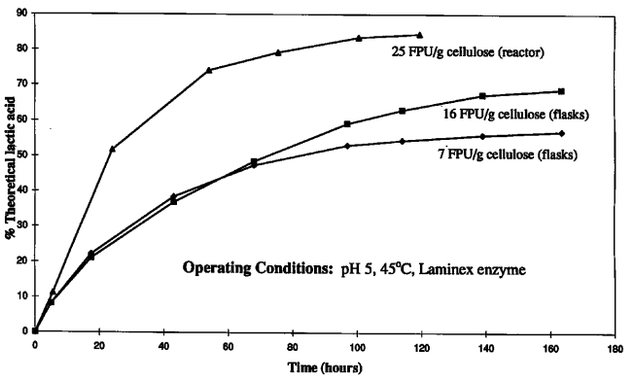

Figure 4 Effect of enzyme loading.

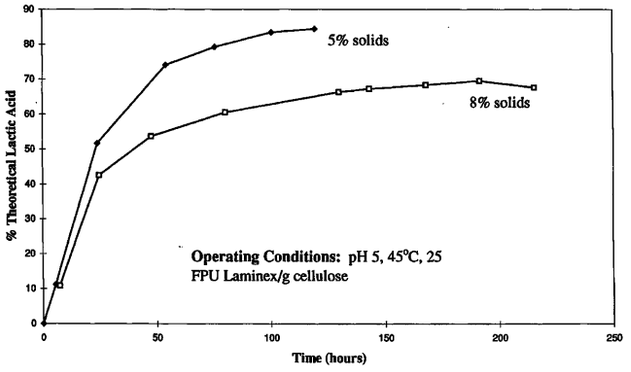

Figure 5 Effect of solids concentration.

a wide-mouth bottle separate from the reactor. The material was then transferred to the sterile reactor within a laminar flow hood. This method allowed better operation of the impeller. At the lower loading of 5% solids, the mixing is facilitated by the relatively more rapid action of the enzyme on the reactor solids. Further work will involve examination of impeller designs for achieving better mixing at higher solids concentrations.

Effect of cellulase preparation

A wide variety of cellulases is available commercially. Our studies investigated the performance of three cellulase preparations obtained from commercial sources. Figure 6 shows that the SSF yields for Laminex, EBT, and COG1 enzymes were 71%, 60%, and 56% of theoretical, respectively, at identical operating conditions. All enzymes were added at 25 FPU g^{-1} cellulose in the shake flask. Laminex showed the best results although it showed the lowest activity in FPU ml^{-1}. It is important to recognize that the enzyme activity assay is performed at pH 4.5 and 50°C, and the SSF at pH 5.0 and 45°C. Because the activity profile for each enzyme preparation is unique, it is conceivable that the actual SSF conditions were more favorable for Laminex than for the other enzymes. To minimize enzyme usage and to meet product cost targets, it is important to identify an inexpensive and efficient enzyme. This study of cellulases is by no means extensive; efforts need to be continued to develop and evaluate alternatives to improve the viability of enzymatic hydrolysis.

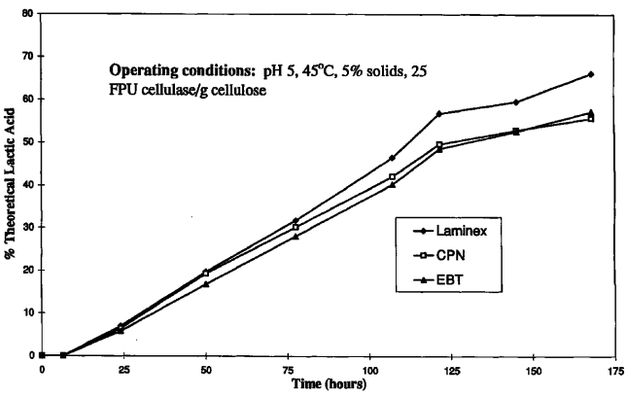

Figure 6 Effect of cellulase preparation.

Carbon balance

An understanding of the pattern of carbon utilization can help identify strategies for maximizing the process yield. Table 1 shows a representative carbon balance for a reactor run at 5% solids, pH 5.0, 42°C, and 25 FPU Laminex g^{-1} cellulose. This run resulted in a product yield of 80% of theoretical. The computed carbon input and output were within 1.3% of each other. Lignin was assumed to remain constant in the calculations. About 66% of the initial carbon in cellulose was converted to lactic acid; a third of the cellulose remained unconverted. More than half the initial xylose and mannose (components of hemicellulose) were metabolized. The release of the sugars from the hemicellulose may be attributed to xylanase activity in the cellulase preparation. The slight increase in galactose and arabinose, present in low concentrations in the feed, is attributed to errors in the composition analysis.

The carbon analysis shows that the carbohydrate consumed, including cellulose, mannose and xylose, is 10.15 g. Theoretical yields from the three consumed components are 1.11, 1.0, and 0.6 g lactic acid per g of substrate, respectively [6]. Therefore, the actual yield of lactic acid based on carbohydrate consumed is 96.5% of theoretical. Xylose metabolism by *L. delbrueckii* results in acetic acid formation as a by-product. We did not detect acetic acid as a product in our analysis probably because of the low concentrations of the compound.

Conclusions

A high product yield of 84% of theoretical lactic acid has been achieved in bench-scale SSF of mixed paper. The pH and temperature of operation to achieve good performance of both the cellulase and the microorganism in the SSF process were identified. The enzyme loading study showed that a decrease in enzyme concentration brought about an undesired reduction in product yield. A reduction in enzyme requirement may be possible if the mixed paper is chemically pretreated or if more efficient enzyme systems are developed. Higher solids loading presents an operational challenge limiting the solids throughput. An increase in solids concentration may be possible through improvements in the reactor design to achieve better mixing. A carbon balance determination showed that about 34% of the cellulose remained unconverted.

Table 1 Carbon balance for SSF of mixed paper to lactic acid (5% solids, pH 5.0, 42°C, 25 FPU Laminex per gram cellulose)

Component	Initial carbon (g)	Final carbon (g)
Cellulose	12.24	4.20
Xylose	1.90	0.52
Mannose	1.33	0.60
Galactose	0.16	0.18
Arabinose	0.19	0.30
Lactic acid	0.0	9.8
Total carbon	15.82	15.62
Lignin	6.30	6.30

$$\% \text{ Difference between input and output carbon} = \frac{15.82-15.62}{15.82} \times 100 = 1.3\%$$

Future research should be directed towards complete utilization of the available carbohydrate for lactic acid production. The present studies were conducted using a rich medium to determine the maximum yields possible without nutrient limitation. Realistically, an inexpensive industrial medium would have to be employed in a large-scale process; future work needs to identify such a growth medium. Research advances in the reduction of enzyme loading, increase in feed solids concentration, and enhancement of cellulose conversion will enable the future development of an economical process. Microorganisms should be developed that can utilize both the hexose and pentose sugars efficiently without by-product acetic acid formation. The presence of coproduct acetic acid would make product purification prohibitively expensive [1]. The effect of lignin breakdown compounds on fermentation and on purification will have to be addressed. Notwithstanding these challenges, our results indicate merit in conducting further studies on the conversion of cellulosic waste feedstocks to chemicals.

Acknowledgements

The authors acknowledge the help of Steve Deutsch and David Johnson in arranging compositional analysis, and of Phillip Planes in conducting laboratory experiments. We thank Charlie Wyman and Barb Goodman for providing guidance throughout the project.

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