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Lactic Acid Production from Repeated-Batch and Simultaneous Saccharification and Fermentation of Cassava Starch Wastewater by Amylolytic *Lactobacillus Plantarum* MSUL 702

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Abstract

The present study is aimed at determining the performance of an amylolytic *Lactobacillus plantarum* MSUL 702 for lactic acid production from the repeated-batch and simultaneous saccharification and fermentation (SSF) of a synthetic cassava starch wastewater (SCW). An ability of the bacteria to treat the SCW in terms of chemical oxygen demand (COD) and total kjeldahl nitrogen (TKN) removal efficiencies was also investigated during the fermentation processes. The SSF experiments were performed for five consecutive batches under a non-sterile condition and at a room temperature. The highest lactic acid concentration and viable lactic acid bacteria at 28.71 g/L and 9.26 log CFU/mL, respectively, were obtained in the 48 h of the first batch fermentation. The highest COD and TKN removal efficiencies at 98% and 85%, respectively, were obtained in the 48 h of the second batch fermentation. The bacteria could retain the high lactic acid production and treatment efficiency up to four consecutive batches.

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Keywords: Lactic acid production, repeated-batch and simultaneous saccharification and fermentation, *Lactobacillus plantarum*, cassava starch wastewater

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1. Introduction

Lactic acid provides a profound application as a chemical feedstock for several industries such as food industry, pharmaceutical industry, leather and textile industries. Recently, the use of lactic acid for production of polylactic acid (PLA) [1], the most well-known bioplastics, leads to a substantial increase in world consumption of lactic acid. However, the price of lactic acid is still too high for its economical use in PLA production. Thus, several attempts have been devoted to develop the cost effective approaches for lactic acid production by seeking for the non-or low cost substrates and the efficient microorganisms, and/or optimizing the fermentation process [2] and [3].

Although the starches have been substituted to the refine sugars for lactic acid production, the high production costs still exist due to the high costs in substrate pretreatment [4]. Currently, an achievement of simultaneous saccharification and fermentation (SSF) of starch to lactic acid by the amylolytic lactic acid bacteria has been noted [3]-[5]. However, these studies were mostly performed by using batch fermentation and under a sterile condition which increased the production costs due to an inoculum preparation and resterilization of the fermentation vessels. In order to reduce the production costs, therefore, the present research is aimed at producing lactic acid from the repeated-batch and simultaneous saccharification and fermentation of a synthetic cassava starch wastewater by an amylolytic *Lactobacillus plantarum* MSUL 702 under a non-sterile condition. The viable lactic acid bacteria (LAB) in the reactor and treatment efficiency of the SSF processes were also investigated.

2. Materials and Methods

2.1. Bacterial Strain and Inoculum Preparation

The *L. plantarum* MSUL 702 was isolated from cassava pulps in cassava starch industry on the basis of ability to produce lactic acid from cassava starch. The bacteria were grown in MRS broth (HiMedia Laboratories, India) supplemented with 1% (w/v) cassava starch. The cultures were incubated at 37 ˚C for 48 h. Cells were harvested by centrifugation at 10,000*g* at 15˚C for 20 min. The harvested cells were resuspended in a sterile phosphate buffer to obtain the initial cell concentration of 1×10^8 CFU/mL and were used as inoculum culture.

2.2. SSF of Cassava Starch to Lactic Acid Experiment

A cylindrical reactor with a working volume of 2 L was operated under a non-sterile condition and at the room temperature. The reactor was fed with a synthetic cassava starch wastewater (SCW), a fermentation medium. The SCW was composed of 0.14 g/L, $FeCl_3$; 0.37 g/L, $CaCl_2.2H_2O$; 0.52 g/L, $MgSO_4.7H_2O$; 0.44 g/L, KH_2PO_4; 1.02 g/L, urea; 2.70 g/L, $NaHCO_3$; 10.00 g/L, $CaCO_3$; 5.00 g/L, yeast extract; and 4%, cassava starch (pH 6.5±0.2). The SCW had the initial COD and TKN concentrations of 7000-8000 mg/L and 14 mg/L, respectively.

The repeated-batch and SSF was started by inoculating the SCW in the reactor with the inoculum culture (4% inoculum size). The mixture was mixed at 250-300 rpm using an agitator (Model OST 20 digital, IKA works, Inc., Germany) to maintain dissolved oxygen at around 1 mg/L. Samples were taken daily and determined for lactic acid and starch concentrations, treatment efficiency, and number of the viable LAB. When the starch in the SCW was exhausted, an agitation was stopped in order to allow the cells to settle down. Then, about 2/3 of the SCW was replaced with a fresh SCW to initiate the next batch. The five consecutive batches were carried out. All SSF experiments were performed in duplicate.

2.3. Analytical Methods

The samples were centrifuged at 10,000*g* at 15˚C for 20 min. The supernatants were analyzed for concentration of lactic acid [6], starch [7], chemical oxygen demand (COD) [8] and total kjeldahl nitrogen (TKN) [8]. The biomass concentration was determined by drying the cells at 80°C until a constant weight was obtained. Then, the dried cells were weighed. The viable LAB were enumerated by the spread plate technique with the use of MRS agar. DO was measured using a DO meter (YSI 200, YSI Inc., USA).

3. Results and Discussion

3.1. Lactic Acid Production from the Repeated-Batch and SSF of SCW

The profiles of lactic acid and starch concentrations of the repeated-batch and SSF of SCW by *L. plantarum* MSUL 702 are shown in Fig. 1. The highest lactic acid concentration at 28.71 g/L was produced in the 48 h of the first batch fermentation while the starch was mostly consumed. The concentration of lactic acid produced was higher than those obtained by [9] which carried out SSF of raw starch by *Streptococcus bovis* 148. It was also higher than the study of [10] and [11] which conducted SSF of cassava starch by *L. amylovorus* ATCC 33620 and *L. rhamnosus*, respectively. However, in the subsequent batches, a reduction of lactic acid production and a longer starch consumption time were observed. The highest lactic acid concentrations in batch 2-5 ranged from 28.39 g/L to 20.29 g/L. These results could be due to the lower cell concentrations in the SCW (Fig. 2). The effects of product inhibition could be another caused of the reduction of lactic acid production [12]. In the fifth batch, lactic acid production was markedly reduced. Thus, the next batch was ceased.

As shown in Fig. 2, the viable LAB and biomass concentration were in concordant to the results of lactic acid production. The highest viable LAB and biomass concentration at 9.26 log CFU/mL and 3,183.33 mg/L, respectively, were obtained in 48 h of the first batch fermentation. A high viable LAB (higher than 8.0 log CFU/mL) was attained until batch 3. After that, the cells were decreased dramatically which might be due to the adverse effects of product inhibition and the contamination since the experiments were conducted under a non-sterile condition. In the 72 h of the fifth batch fermentation, a residual starch in the SCW was presence and the highest viable LAB was at

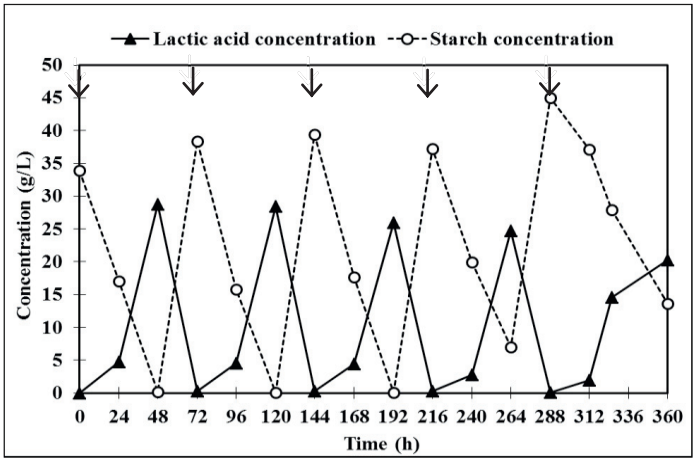

Fig. 1. Lactic acid production from the repeated-batch and SSF of SCW by L. plantarum MSUL 702. The arrows indicate the initial time

of each batch.

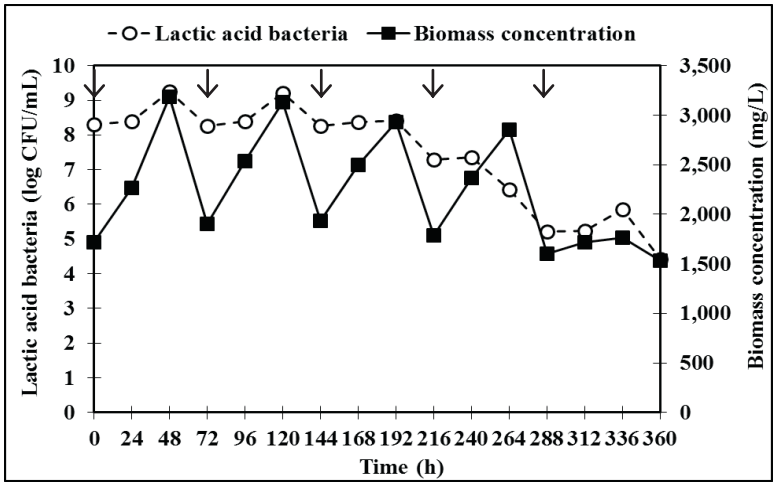

Fig. 2. The number of viable LAB and biomass concentration in the repeated-batch and SSF of SCW by L. plantarum MSUL 702. The arrows indicate the initial time of each batch.

5.87 log CFU/mL which produced lactic acid concentration at only 20.29 g/L. These results could infer a decrease in cell activity as reported by [13]. Therefore, to improve lactic acid production efficiency in the repeated-batch and SSF of SCW by *L. plantarum* MSUL 702, cell immobilization should be studied to increase the initial cell concentration in the subsequent batches. In addition, further study in the use of lactic acid bacterial cells obtained from the drainage of fermentation process as probiotics in animal feed is recommended for environmental friendly production of lactic acid.

3.2. Treatment of SCW in the Repeated-Batch and SSF

The repeated-batch and SSF by *L. plantarum* MSUL 702 showed a high performance in treating the SCW (Fig. 3). In the 48 h of the second batch fermentation, the COD and TKN concentrations of the SCW were 872.9 mg/L and 2.1 mg/L (Data not shown), respectively, which resulted in the highest COD and TKN removal efficiencies at 98% and 85%, respectively.

4. Conclusions

The present study reveals a high performance of amylolytic *L. plantarum* MSUL 702 in production of lactic acid from the synthetic cassava starch wastewater via the repeated-batch and SSF under a non-sterile condition. A high treatment efficiency of SCW was also obtained. The viable LAB retained a high metabolic activity in lactic acid production for at least four consecutive batches.

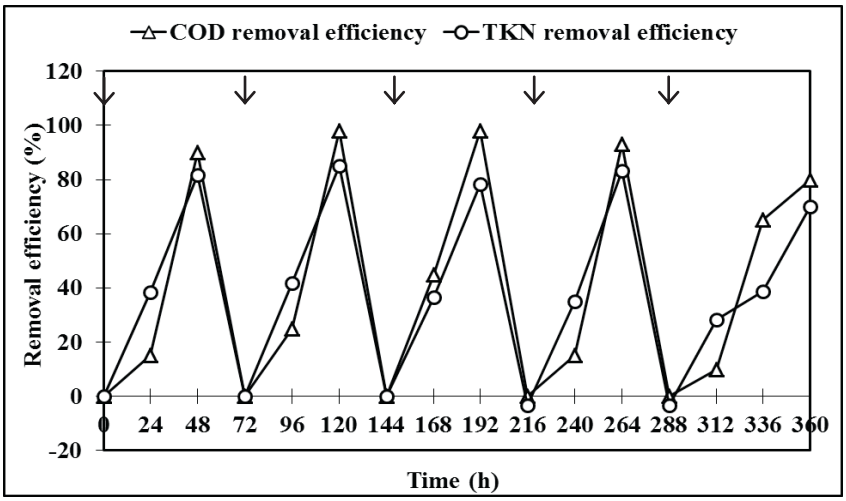

Fig. 3. Treatment of SCW in the repeated-batch and SSF to produce lactic acid by L. plantarum MSUL 702. The arrows indicate the initial time of each batch.

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