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Continuous ethanol production by the synchronous saccharification and fermentation using food wastes

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Abstract−The synchronous saccharification and fermentation (SSF) by continuous fill and draw method was investigated in order to develop a continuous ethanol fermentation process using the food wastes (FW) available among Korea's organic wastes. The activity of the hydrolytic enzymes was maintained constantly in the continuous culture by their intermittent addition together with medium exchange. The concentrations of reducing sugar in the culture were maintained at a steady state by regulating supplemented enzyme concentration and exchange rate of medium, reflecting on the consumption rate of reducing sugar caused by the fermentation. When the temperature of the SSF was maintained at the fixation of 35 °C, which enabled us to perform both enzymatic hydrolysis and enzyme fermentation simultaneously, the rate of reducing sugar consumption was 3.61 g/L-hr. For the enzymatic saccharification of FW, when 0.01 BGU as Viscozyme/g-FW and 0.05 AGU as Spirizyme Plus/g-FW were used, the production rate of reducing sugar was 3.93 g/L-hr, indicating a little higher rate of production than that of consumption. A decompression device with ethanol condensing ability was used to continuously pull out ethanol from the culture broth at –600 mmHg, where the ethanol evaporation ability would be maximized and the water evaporation minimized during the process. As a result of the continuous SSF performance, the reducing sugar concentration was maintained at around 30 g/L. The amylase activity was maintained at 8.93±2.17 U/mL. During a 352 hour culture, the whole ethanol productivity was 2.24 g/L-hr, indicating a considerable productivity compared with the other result reported in the continuous SSF.

Key words: Continuous Ethanol Fermentation, Synchronous Saccharification and Fermentation (SSF), Bioethanol, Food Wastes, Decompression

INTRODUCTION

The current demand for energy is increasing, with fossil fuels, which are non-renewable energy sources, being overused. Also, fossil fuels destroy the natural and living environment and contribute to the greenhouse effect and ozone depletion. Therefore, a pure energy source that could be used as a substitute for fossil fuels should be urgently exploited. Due to the future exhaustion of petroleum, bioethanol as a substitute source energy is urgently required [1].

The raw material used for the bioethanol fermentation initially employed grains during the first generation, wood-like materials during the second generation and ocean biomass, such as algae, during the third generation.

On the other hand, 14,026 ton/day of food wastes (FW) is generated in Korea [2] that contains many organic compounds, cellulose, hemicelluloses, starch and protein, which are available as carbon and energy sources for the fermentation industry like bioethanol.

In general, the batch process in fermentation is a multi-vessel process that allows flexible operation and easy control over the process. The batch fermentation is characterized by low productivity with intensive labor [3]. In addition, elaborate preparatory procedures are needed, and because of the discontinuous start up and shut down operations, high labor costs are incurred. This inherent disadvantage and the low productivity offered by the batch process have led many commercial operators to consider the other fermentation methods like a continuous culture mode [4].

The synchronous saccharification and fermentation (SSF) process is well known as the most appropriate process for the economic production of ethanol. The SSF is preferred over separate hydrolysis and fermentation process, since its operation can be performed in the same tank, resulting in lower facility and energy costs, higher ethanol productivity, shorter processing time and lower enzyme usage [5,6].

To improve ethanol productivity, many researchers have focused on recombination of bioethanol producing strain and amylase-producing gene or development of new genes, such as MSN2, DOG1, HAL1 and INO1, which contributed increase efficiency of ethanol production and economic feasibility [7]. However, the stability of the recombinant gene has not been identified yet.

Holzberg reported a product inhibition of ethanol with a linear relationship [8] and many analogous results about the inhibition phenomena have been known. Therefore, to minimize the product inhibition the ethanol produced during the fermentation needs to be eliminated from the culture. A decompression device was considered to continuously pull out ethanol from the culture broth in this research.

In this study, to perform the continuous ethanol production efficiently, a fill and draw mode using the synchronous saccharification and fermentation (SSF) process was employed by partial exchange of medium. In addition, a decompression and distillation apparatus

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drawing out the ethanol was equipped for the continuous SSF culture system to minimize the product inhibition of ethanol. Food wastes as a medium for the ethanol culture were employed to cut down on the production cost and the utilization could be an eco-friendly alternative treatment method in Korea.

MATERIALS AND METHODS

1. Microorganism and Media

Saccharomyces italicus KJ was used for the ethanol fermentation [9], with YM medium (yeast extract 3.0 g/L, malt extract 3.0 g/L, bacto peptone 5.0 g/L, glucose 10.0 g/L, agar 15.0 g/L) used as the solid pre-culture medium. The food wastes used in this experiment were obtained from the 1st student cafeteria at Chonnam National University, Korea, and homogeneously ground twice with a grinder (WP650A, Wonpool). Generally, the compositions of food wastes are known as grains 16±2%, vegetables 51±2%, fruit 14±2%, and meats 19±2% (Waste Management Act in Korea). The elemental composition of food wastes used in this research was 2.94% nitrogen and 51.03% carbon via an elementary analysis. The ground food wastes were divided into dozens of parts and maintained in a freezer at –20 ºC, and then later supplemented into the SSF; thereby, food wastes of the same composition could be used during the entire continuous SSF experiment.

2. Determination of Enzyme Concentration for FW Saccharification

In our previous work, the optimum fermentable temperature of the strain *S. italicus* KJ in the SSF process using food wastes was found to be 35 ºC [9], so the saccharification reaction of food wastes was performed at 35 ºC. To maintain the reducing sugar consumption and production rates at similar rates at 35 ºC, the appropriate selection of enzymes and accurate enzyme activities was required in the continuous SSF process. One enzyme group, composed of Termamyl 120 L and Spirizyme Plus, and another of Viscozyme and Spirizyme Plus, was examined to evaluate the saccharification efficiency of food wastes. Termamyl 120 L (Novozyme) is an endoamylase, which hydrolyzes 1, 4-alpha-glucosidic linkages, and Spirizyme Plus (Novozyme) is an amyloglucosidase (glucoamylase), which hydrolyzes 1, 4- and 1, 6-alpha linkages in liquefied starch-containing substrates. Also, Viscozyme (Novozyme) is a multi-enzyme of beta-glucanase, xylanase, cellulase and hemicellulase.

3. Determination of Optimal Concentrations of Saccharification Enzymes

Termamyl 120 L and Spirizyme Plus enzymes are usually used in Korean ethanol fermentation factories [10]. 200 g of food wastes (80% water contents) and the enzyme of Termamyl 120 L (the final concentration 0.01 KNU/g-FW; KNU: Kilo Novo alpha-amylase Unit) were mixed and liquefied for 2 hours. Spirizyme Plus (the final concentration 0.015, 0.029 and 0.035 AGU/g-FW; AGU: Amyloglucosidase Units) was then added into the reactants and saccharified for a further 8 hours. When using Viscozyme and Spirizyme Plus, 200 g of food wastes (80% water contents) and the enzymes of Viscozyme (the final concentration 0.004, 0.008 and 0.010 BGU/g-FW; BGU: Beta-glucanase Unit) and Spirizyme Plus (the final concentration 0.02, 0.04 and 0.052 AGU/g-FW) were mixed and saccharified for 8 hours. Sampling was then performed every 2 hours and reducing sugar concentrations were determined by using the DNS method [11].

4. Determination of Decompression Condition in SSF

A decompression and distillation method was introduced to reduce product inhibition during the SSF ethanol fermentation. To determine the optimum decompression conditions, the ethanol evaporation rate from the broth and the overflow rate to the outlet of the evaporator were investigated under various decompression conditions, using 50 g/L of ethanol solution and 50 g-ethanol/L of SFW medium to imitate similar concentration of the ethanol fermentation broth.

In the SSF process, the ethanol concentration in the culture reached 50 g/L within 24 hours of fermentation. Each 42.5 g/L of ethanol solution was evaporated and enriched at 35 ºC for 1 hour under the decompression conditions of –400, –500, –600 or –700 mmHg (decompression pump: SMC ZSE30, Seoul Union, Korea). Also, 41.6 g/L ethanol-containing SFW was evaporated and enriched under the same conditions. In the enrichment apparatus, a 15% ethanol solution was used as a cooling solute and set at 0 ºC (cooling system: LCB-R08, Lab Tech, Korea).

5. Continuous Ethanol Production in the SSF by Fill and Draw

Continuous ethanol fermentation in the SSF process by the fill and draw mode was performed in the evaporation and condensation system. As shown in Fig. 1, the SSF was performed in the reactor of an evaporator equipped with a water bath of 35 ºC. The culture reactor with a working volume of 250 mL connected into the evaporator was rotated at 100 rpm. The ethanol evaporated from the continuous SSF was collected in a condensing system. For the evaporation under the constant decompression in the continuous SSF, a regulable decompression pump (SMC ZSE30, Seoul Union, Korea) was employed to the evaporation and condensation system.

Initially, 200 g food wastes (80% water contents) and 50 mL of Viscozyme (0.010 BGU/g-FW or 0.005 U/mL as final concentration of α-amylase) and Spirizyme Plus (0.052 AGU/g-FW or 0.655 U/mL as final concentration of α-amylase) in 50 mM sodium acetate buffer (pH 5.0) were mixed for an 8 hour saccharification reaction. Then 5% of pre-cultured *S. italicus* KJ was inoculated into the culture broth. After 20 hrs of fermentation, 80 g of food wastes was added to the SSF medium and the same amount of culture broth was pulled out at 12 hr intervals. The saccharifying enzymes (Viscozyme of 0.005 U/mL and Spirizyme Plus of 0.655 U/mL as final

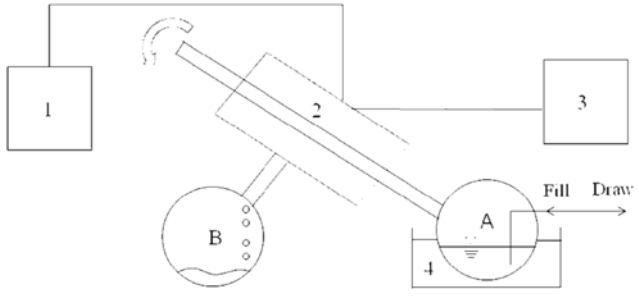

Fig. 1. Schematic diagram of the SSF process employing the fill and draw mode used in this study.
1. Decompression pump
2. Evaporator
3. Condensing system
4. Constant-temperature water bath
A. Culture flask
B. Ethanol condensing flask

concentration of α-amylase) were added into the culture at 24 hour intervals. In the 24 hour culture, ethanol enrichment by decompression and distillation was started at −600 mmHg, with 15% ethanol set at 0 °C used as the cooling solute (Fig. 1).

6. Analytical Method

α-amylase activity was monitored as a representative activity of the hydrolysis enzymes in the continuous SSF. The amylase activity in the supernatant was analyzed via the amount of reducing sugars produced in 30 min from 0.2 mL of culture broth with 0.8 mL of 1% soluble starch. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol glucose per 1 minute, and was expressed as U/mL. The reducing sugar concentration was also determined using the dinitrosalicylic acid reagent (DNS) method [11]. The ethanol concentration was determined by gas chromatography (GC, HP 5890) with an FID and HP-FFAP capillary column (50 m/0.32 mm/0.5 μm). The temperatures of the oven, injector and detector were 60, 200 and 200 °C, respectively. Pretreatment of each ethanol culture broth was firstly centrifuged at 12,000 rpm for 10 min, and then ethanol solution of the supernatant was filtered with 0.45 μm syringe filter and diluted with acetone appropriately. Then, the prepared ethanol solution of 1 μl was injected into the gas chromatography.

RESULTS AND DISCUSSION

1. Effect of Enzyme Concentration on Saccharification of FW

To maintain reducing sugar consumption and production rates constantly, the enzyme concentration was first examined to evaluate the rate of reducing sugar production at the fixation of 35 °C, which enabled us to perform ethanol fermentation and enzymatic saccharification simultaneously as described in our previous work [9].

When 0.015, 0.029 and 0.035 AGU/g-FW of Spirizyme Plus, after liquefying food wastes with Termamyl 120 L of 0.01 KNU/g-FW, were mixed and saccharified for 8 hours, the rates of reducing sugar production were 2.21, 2.70 and 2.66 g/L, respectively, as shown in Fig. 2.

Also, when 0.004, 0.008 and 0.010 BGU/g-FW of Viscozyme and 0.02, 0.04 and 0.052 AGU/g-FW of Spirizyme Plus were mixed with food wastes and saccharified for 8 hrs, the rates of reducing sugar production were 2.75, 2.98 and 3.93 g/L, respectively (Fig. 2). On the other hand, the rate of reducing sugar consumption by the ethanol fermentation strain was - 3.61 g/L-hr, as shown in Fig. 3. For the enzymatic saccharification of food wastes, when 0.01 BGU/g-FW of Viscozyme and 0.05 AGU/g-FW of Spirizyme Plus were used, the rate of reducing sugar production was 3.93 g/L-hr, indicating a slightly higher rate of production than that of consumption. Therefore, the above combination of Viscozyme and Spirizyme Plus was determined as the optimum condition for saccharifying food wastes in the continuous SSF process.

2. Determination of the Decompression Condition

In this study, a decompression and distillation technique was equipped to reduce product inhibition in the continuous SSF process. When each 42.5 g/L ethanol solution was enriched at 35 °C for 1 hour, under decompression conditions of −400, −500, −600 and −700 mmHg, the rates of ethanol evaporation were 6.14, 6.53, 8.04 and 26.93 g/L-hr, respectively, as shown in Table 1. Also, when 41.6 g-ethanol/L-SFW containing medium was enriched under the same conditions, the rates of ethanol evaporation rate were 0.67, 7.90, 10.55 and 26.79 g/L-hr, respectively, as shown in Table 1.

Under the decompression conditions of −400 and −500 mmHg, all the ethanol distillated flowed over, without conversion to condensed ethanol in both ethanol solution and ethanol-containing SFW medium (Table 1). The ethanol overflow rate with the SFW medium

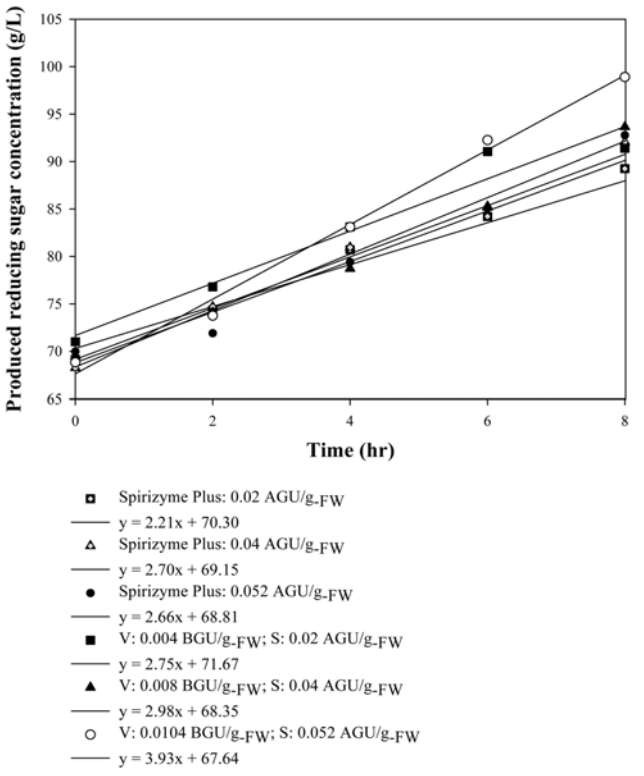

Fig. 2. The production rates of reducing sugar according to the concentrations of Spirizyme Plus and another set of Viscozyme (v) and Spirizyme Plus (s) after liquefying food wastes with Termamyl 120 L at 35 °C.

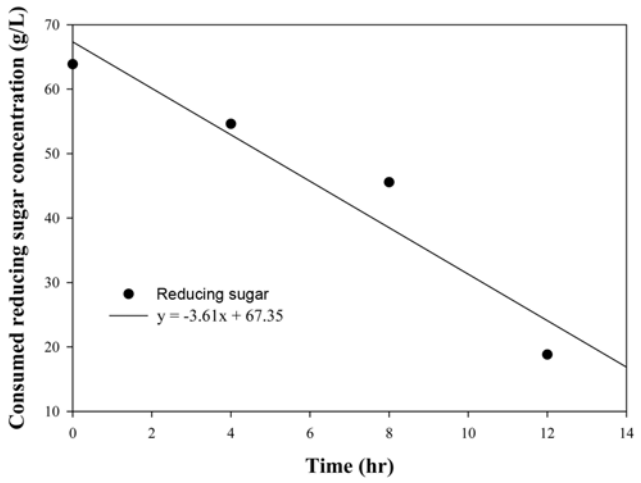

Fig. 3. The consumption rates of reducing sugar in the exponential phase of batch ethanol fermentation using saccharified food wastes (SFW) medium at 35 °C.

Table 1. The evaporation and condensing experiments of ethanol solution (42.5 g-ethanol/L-H_2O) and ethanol containing SFW medium (41.6 g-ethanol/L-SFW) under the various decompression conditions at 35 °C for 1 hr

Pressure (mmHg)	Solution	Remaining Conc. (g/L)	Remaining Vol. (mL)	Condensed Conc. (g/L)	Condensed Vol. (mL)	Overflow rate (g/L-hr)	Evaporation rate (g/L-hr)
−400	E	37.1	98	--	--	6.14	6.14
	E+SFW	41.6	246	--	--	0.67	0.67
−500	E	36.7	98	--	--	6.53	6.53
	E+SFW	35.4	238	--	--	7.90	7.90
−600	E	35.9	96	96.5	1.0	7.10	8.04
	E+SFW	31.5	245	108.4	0.94	10.14	10.55
−700	E	17.5	89	50.4	4.6	24.60	26.93
	E+SFW	16.1	230	81.7	3.6	25.60	26.79

E: Ethanol solution (42.5 g-ethanol/L-H_2O); E+SFW: Ethanol+SFW medium (41.6 g-ethanol/L-SFW medium). The concentrations of ethanol remaining in the ethanol solution after evaporation (remaining conc.) and its volume (remaining vol.); the concentration of ethanol condensed (condensed conc.) and its volume (condensed vol.); ethanol in exhausted gas which is not condensed (overflow rate); amount of condensed and overflowed ethanol (evaporation rate), that is, elimination rate of ethanol continuously pulling out from the ethanol containing solution or the culture broth

at −600 mmHg was 10.14 g/L-hr, indicating that a large part of distillated ethanol was lost through the decompression device, even though the 15% ethanol solution was used as a cooling solute at 0 °C.

At −600 mmHg, a small amount of the distilled ethanol accumulated in the condensing vessel in both the ethanol solution and SFW medium. Therefore, the optimum decompression condition for the continuous SSF was determined to be −600 mmHg.

3. Ethanol Production in the Continuous SSF by Fill and Draw

In the evaporator apparatus with a working volume of 250 mL, 200 g of ground food wastes, Viscozyme of 0.005 U/mL and Spirizyme Plus of 0.655 U/mL as α-amylase were initially reacted for 8 hours for the saccharification, and then 5% of *S. italicus* KJ was inoculated into the reactant. After 20 hours of fermentation, 80 g of ground food wastes was added and the same amount of the culture broth was pulled out at 12 hrs intervals, with the enzyme solution added at 24 hour intervals. After 24 hours, the ethanol decompression enrichment was started at −600 mmHg. In the continuous production of ethanol by the fill and draw mode in the SSF process, the reducing sugar concentration was maintained at around 30 g/L, and the amylase activity at 8.93±2.17 U/mL.

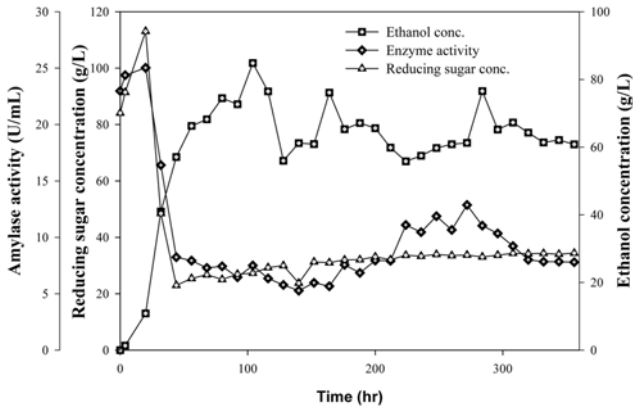

Fig. 4. Changes of the reducing sugar (RS) concentration, amylase activity and ethanol concentration in the culture broth (CB) during the continuous SSF process by fill and draw.

The whole production of ethanol in the SSF process was calculated in three parts: first, that in the condensed vessel, 0.31 g/L-hr; second, the rate of ethanol production in the culture broth, which was pulled out twice every day, 1.74 g/L-hr; and third, the rate of ethanol production in the remaining culture broth at the end of the SSF, 0.19 g/L-hr. As a result, for 352 hours of continuous ethanol fermentation culture, the total productivity of ethanol was calculated to be 2.24 g/L-hr (Fig. 4).

Our SSF result using food wastes was comparable to that of Svetlana et al. even using corn meal. Svetlana et al. performed the production of bioethanol from corn meal, using simultaneous enzymatic saccharification and fermentation with immobilized cells, and reported volumetric ethanol productivity of 2.13 g/L-hr [12], proving a little higher productivity of 2.24 g/L-hr obtained in this study.

CONCLUSIONS

A continuous ethanol SSF process by fills and draw, with continuous distillation and condensation, was examined and the process used food wastes generated in Korea in order to cut down on the production cost. For the continuous SSF process using food wastes, the rate of reducing sugar production was 3.93 g/L-hr at 35 °C, when 0.01 BGU/g-FW of Viscozyme, containing beta-glucanase, xylanase, cellulase and hemicellulase, and 0.05 AGU/g-FW of Spirizyme Plus, containing amyloglucosidase, were used in enzymatic saccharification of food wastes. At 35 °C that enable fermentation and saccharification, the rate of reducing sugar consumption caused by ethanol fermentation was 3.61 g/L-hr. A decompression device in the SSF process, for reducing fermentation inhibition caused by the ethanol produced, was used to continuously pull out ethanol from the culture broth, and its optimal decompression condition was determined to be −600 mmHg. During 352 hours of continuous SSF ethanol fermentation, the whole productivity of ethanol was 2.24 g/L-hr, indicating a considerable productivity compared to the other known continuous SSF operations.

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