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A CONTINUOUS ALCOHOL FERMENTATION BY *KLUYVEROMYCES FRAGILIS* USING JERUSALEM ARTICHOKE

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Abstract—In this study, a continuous fermentation of inulin in the tubers of Jerusalem artichoke (JA) for alcohol production was investigated. Experiments were also conducted on the fermentation of mashed JA tubers, without extracting the juice. In a continuous fermentation of the juice of JA tubers, alcohol productivity was increased by 3.8 times as compared with that obtained in a batch fermentation. The liquefaction of mashed JA tubers by enzymes, pectinase and cellulase followed by fermentation of liquefied solution by *K. fragilis* was found as effective as direct fermentation of the juice. The results of this study are expected to provide valuable information in the utilization of Jarusalem artichoke for ethanol production.

INTRODUCTION

The alcohol production from the carbohydrates in the energy crops has been drawing much interests. Jerusalem artichoke is known as one of the potential energy crops which contains a substantial amount of carbohydrate in the form of inulin. The inulin content in the JA tubers is approximately 15-20% (w/w) [1].

The processes for the conversion of inulin to ethanol include; saccharification by an acid or an enzyme, the inulase, followed by alcohol fermentation [2, 3], and the direct conversion to ethanol by a microorganism capable of both inulase production and fermentation [4, 5]. Studies have been conducted on the conversion of inulin in the juice extracted from JA tubers to ethanol by *Kluyveromyces fragilis* and also on the various factors influencing the alcohol tolerence of *K. fragilis* in a batch fermentation [6]. Over 85% of inulin has been converted to ethanol and the alcohol tolerance of yeast strains has been increased by aeration or by addition of high content ergosterol and unsaturated fatty acids, linoleic and oleic acids, to the medium due to higher alcohol diffusion rate through the cell membrane into the medium [7, 8].

While attempts have been made to improve the fermentation condition in a batch system, higher alcohol productivity has been obtained in continuous fermentation system [9]. Especially, by employing cell recycle [10] and immobilization systems [11, 12], the alcohol productivity has been substantially improved. The alcohol productivity obtained from the juice of JA tubers by Margaritis et al. was ten times as high as that in a free cell system [13, 14].

In this study the fermentation in a continuous system using the juice of JA tubers was examined. An investigation was also made on the fermentation of the mashed JA tubers without extracting the juice. With this process, not only one of the process steps, the extraction of juice, could be eliminated but the utilization of raw material could be enhanced.

The alcohol productivity was found substantially increased in a continuous system and the liquefaction of mashed tubers by adding small quantities of enzymes, pectinase and cellulase with subsequent alcohol fermentation *K. fragilis* in a batch system was found as effective as direct fermentation of the juice of JA tubers.

Since high alcohol content and high productivity are required in the economic fuel alcohol production for industrial use, the results of this study would provide valuable information in the process design for the alcohol production from JA tubers.

MATERIALS AND METHODS

The organism used in the experiment was *K. fragilis* CBS 1555. In the batch and continuous fermentation experiments, the medium was prepared by extracting the juice of JA tubers which were cooked for 30 min at 121℃. The pH was adjusted to 5.5 using conc. $-H_3PO_4$ and the medium was sterilized in an autoclave at 121℃ for 15 min. After sterilization, 1 ml of antifoam-

ing agent was added. The total sugar concentration was 180 g/*l*. The experiments were performed in a 1.5 liter jar fermentor at an aeration rate of 0.05 vvm and at 30℃ with the agitation speed of 300 rpm. The range of dilution rate was 0.02-0.35 hr^{-1}. In the experiments with the mashed JA tubers, the medium was prepared by mashing the tubers which were cooked at the conditions specified in the continuous experiments, with 200 ml of water per 100g tubers added. The total sugar concentration was 160 g/*l*. For the liquefaction, the enzymes, pectinase and/or cellulase were added and the enzymatic liquefaction was carried out in a batch fermentor at 50℃ for 35 hrs. The concentration of alcohol was measured by gas chromatography and the cell concentration was determined by measuring the number of cells using Thoma cell which was then converted to the concentration in g/*l*.

Using the data obtained in a batch fermentation the parameter for the continuous operation could be estimated. A simple single-stage chemostat model equation is,

$$\frac{dx}{dt} = \mu X - \frac{F}{V}X, \tag{1}$$

where X=cell mass concentration (g/*l*), μ=specific growth rate (hr^{-1}), F=volumetric flow rate (1/hr), V=reactor volume (1) and t=time (hr).
At steady state, dX/dt=0 in Eq. (1) and hence, the dilution rate D is given as,

$$\mu = D = \frac{F}{V}. \tag{2}$$

The ethanol productivity of continuous system, P is given as,

$$P = D \cdot p. \tag{3}$$

where p=ethanol concentration (g/*l*)
As the specific growth rate μ is defined as

$$\mu = \frac{1}{X}\frac{dX}{dt}, \text{ or } \frac{dX}{dt} = \mu X = DX \tag{4}$$

at steady state, the dilution rate, D can be obtained from dX/dt vs. X plot of the batch experimental data. The cell concentration in a continuous system hence, can be estimated from this plot for different dilution rates. The alcohol concentration could similarly be estimated.

RESULTS AND DISCUSSIONS

1. Batch fermentation

The cell growth, ethanol production and sugar utilization curves in the batch fermentation under aerobic conditions are shown in Figure 1. The maximum specific growth rate, μ_{max}, was 0.360 hr^{-1} and maximum cell concentration reached 9.7 g/*l* in 26 hrs. The overall ethanol productivity was 3.3 g EtOH/1-hr at the maximum ethanol concentration, which was 73 g/*l* in 22 hrs.

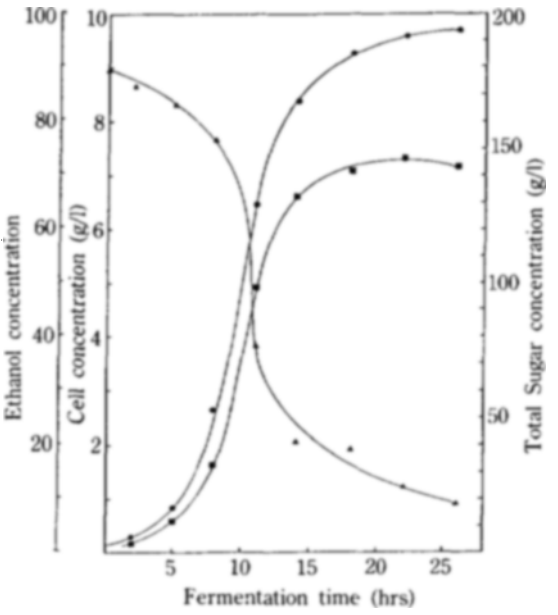

Fig. 1. Batch fermentation of *Kluyveromyces fragilis* in J.A tuber juice under aerobic condition.

2. Continuous fermentation

Using the batch fermentation data the steady state cell and the ethanol concentration for different dilution rates were estimated based on the model for the continuous system and compared with the experimental data.

Shown in Figure 2 are the growth rates vs. cell concentration curve from batch data along with straight lines of dilution rates. A similar plot for the ethanol production is shown in Figure 3. From these plots, the changes in cell concentration and ethanol concentration with dilution rates were constructed (Fig. 4) and the comparisons with the experimental data were made.

Both the experimental data and the estimation from the model show the typical characteristics observed in the complex media containing the juice of JA tubers; the cell and the alcohol concentrations decrease with the dilution rates, which is believed due to the deficiency in the actual limiting nutrients which are rapidly consumed due to high cell growth rate [17].

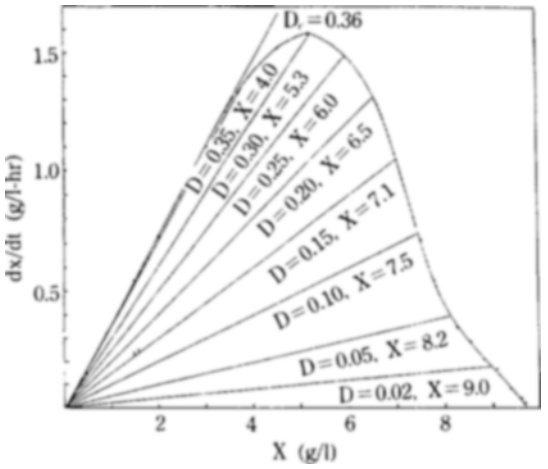

Fig. 2. Plot of cell growth rate vs. cell concentration.

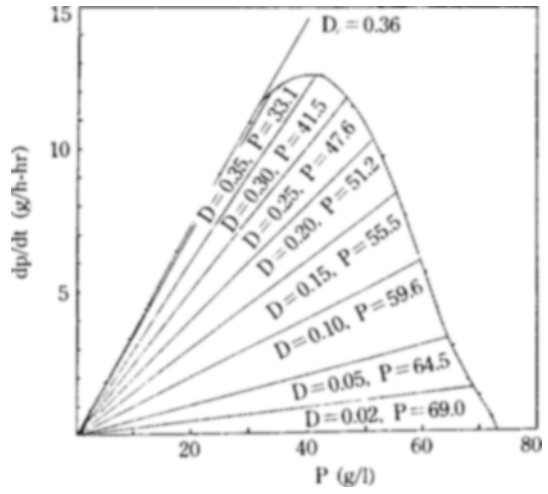

Fig. 3. Plot of ethanol production rate vs. ethanol concentration.

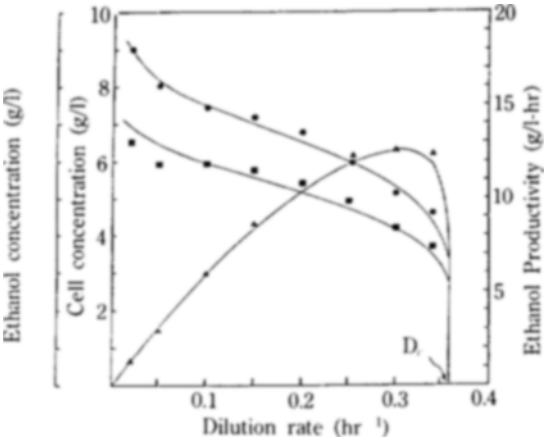

Fig. 4. Plot of cell concentration (●), ethanol concentration (■) and ethanol productivity (▲) vs. dilution rate.

——: Calculated value, ●■▲: Experimental values.

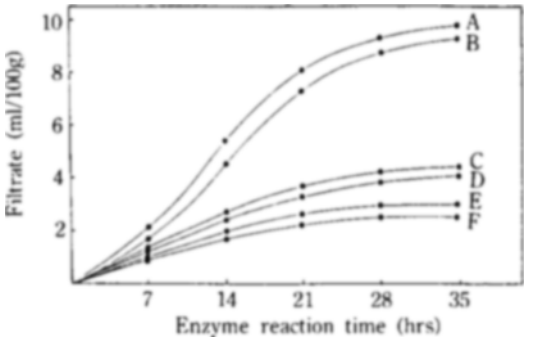

Fig. 5. Liquefaction of mashed J.A tubers by enzyme treatment at 50°C.

A: Cellulase 1.0% + Pectinase 1.0%
B: Cellulase 0.5% + Pectinase 0.5%
C: Cellulase 1.0% or Pectinase 1.0%
D: Cellulase 0.1% + Pectinase 0.1%
E: Cellulase 0.05% + Pectinase 0.05%
F: Cellulase 0.01% + Pectinase 0.01%

The high cell concentration at the dilution rates lower than 0.1 hr^{-1} is attributed to the fact that the cell growth continues by consuming the alcohol as a carbon source when the reducing sugar becomes growth limiting [18].

The ethanol productivity in a continuous fermentation was approximately 12.5 g EtOH/1·hr at the dilution rate of 0.3 hr^{-1} (Fig. 4) and this value was substantially higher than that obtained in a batch system, 3.3 g EtOH/1·hr.

3. Fermentation of mashed tubers

For the economic alcohol fermentation an attempt was made to use the mashed tubers as the substrate. This would not only eliminate one of the process steps, but enhance the utilization of the inulin in the tubers, by preventing the loss of inulin in the solid. When the mashed tubers without liquefaction was used directly for the fermentation however, only 17-22% of total sugar has been converted to ethanol. The low conversion is believed due to slow cell growth because of the physical limitations imposed by the pulp-like nature of mashed tubers.

The mashed tubers, hence has been treated by the enzyme (s) of 0.05-2.0% (v/v) to 50 g of mashed tubers

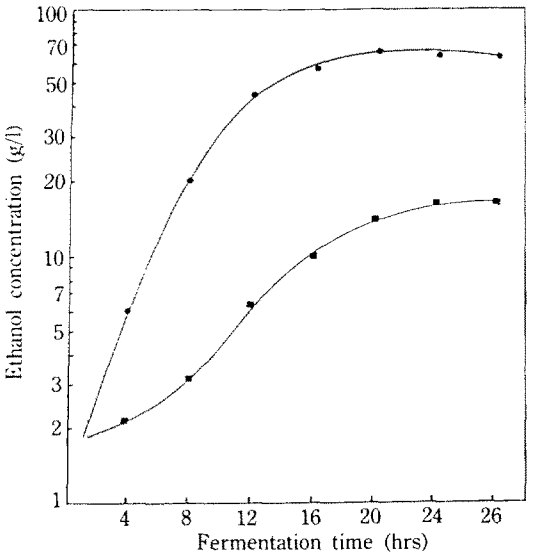

Fig. 6. Ethanol production during the fermentation time in the liquefaction of mashed tuber by enzyme (●) and in the non-liquefaction of mashed tuber (■).

and the liquefaction was carried out at 50℃ for 35 hrs in a batch system, in order to determine the condition and the amount of enzyme (s) needed for highest liquefaction.

Samples were take at each 7 hrs and the filtrate was measured. Shown in Figure 5 is the amount of the mashed tubers liquefied by enzymes, pectinase and/or cellulase. The liquefaction was highest when the mashed tubers was treated with both pectinase and cellulase, 1% (v/v) each.

The subsequent fermentation was carried out by *K. fragilis* in a batch system under aerobic condition and at the same conditions as in the fermentation of the juice. As shown in Figure 6 the ethanol concentration of liquefied and non-liquefied solutions after 24 hrs of fermentation were 64.0 g/*l* and 16.5 g/*l*, respectively. The fermentability (sugar converted to ethanol/total sugar×100) of liquefied solution was 85% which was comparable to the result from the fermentation of juice, while that of non-liquefied solution was 22%.

CONCLUSIONS

Studies on the continuous fermentation of the juice of Jerusalem artichoke tubers and on the batch fermentation of the mashed JA tubers led to the following conclusions;

1. The cell and the alcohol concentrations estimated by a simple single-stage chemostat model were in good agreement with the experimental data.

2. The maximum dilution rate was 0.360 hr^{-1} in both the cell growth and the ethanol production, and the ethanol productivity was 12.5 g EtOH/l·hr which was substantially higher than that in a batch system, 3.3 g EtOH/l·hr.

3. The highest liquefaction of the mashed JA tubers was obtained when the enzymes, pectinase and cellulase [1%(v/v) each] were added, and in the subsequent fermentation of the liquefied solution by *K. fragilis*, over 85% of the inulin in the tubers was converted to ethanol which was comparable to that obtained from the juice of JA tubers in a batch system.

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