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Alcohol Production from Whey in Batch and Continuous Culture of *Kluyveromyces fragilis*.

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In order to develop the whey beverage, we examined the optimum conditions for alcohol fermentation by *Kluyveromyces fragilis* ATCC 46537. The optimum conditions for alcohol production by *K. fragilis* ATCC 46537 were as follows; pH 4.5, 30°C, with a supplement of 50 g/l of lactose. To develop a continuous production of alcohol from whey, we compared batch fermentation with continuous fermentation in conjunction with UF system. Batch fermentation produced 11.0 g/l of alcohol, whereas pseudocontinuous and continuous fermentation with UF system produced 8.5 g/l of alcohol. To increase the alcohol production, we added 50 g/l of lactose to both fermentations. Batch fermentation with lactose supplement produced 15.7 g/l of alcohol and continuous fermentation with lactose supplement in conjunction with UF system produced 15.0 g/l of alcohol. These results clearly demonstrate that the UF system can be used to increase the alcohol production from whey, supplemented with exogenous lactose.

Whey is the by-product of the production of cheese or casein (85~90% of raw milk). Although whey is produced in large quantities, only about half of it is utilized while the remaining half is disposed (9). Therefore, searching for new technologies of whey utilization is desirable to reduce the disposal cost. In addition, since whey is rich in nutrients, that include whey protein, lactose, vitamins and minerals, there is a great potential to process the whey for food production (4, 9).

To develop whey beverage, it is prerequisite that one prevents the precipitation of protein (11, 14) and removes the undesirable whey flavor (16, 17). Proteins and other components can be separated by the methods of ultrafiltration (UF), ion exchange and centrifugation (5, 6, 18). Ultrafiltration is being used increasingly to process milk prior to soft-cheese making and to fractionate whey into protein concentrate and a lactose-rich permeate. In addition, in order to prevent lactose intolerance (7, 19), a large amount of lactose needs to be eliminated or alternatively, an enzyme or a microorganism can be used to reduce the lactose content (1, 2, 15). Among the solutions, one way is to ferment the lactose portion of whey to produce ethanol (8).

Previously, we have examined optimum conditions for the lactic acid fermentation from whey with lactic acid bacteria, and for lactose hydrolysis with β-D-galactosidase (3). Also, we have attempted to use the alcohol fermenting strains of yeast, especially *K. fragilis* to produce whey alcoholic beverage. In this study, we extended the latter effort to determine optimum conditions for alcohol production from whey by pseudocontinuous and continous fermentation along with UF system using *K. fragilis*.

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Key words: ultrafiltration (UF), whey, batch, pseudocontinuous & continuous fermentation

MATERIALS AND METHODS

Strains

K. fragilis ATCC 28244, *K. fragilis* ATCC 46537 and *S. cerevisiae* IFO 2346 were used. These strains were maintained in YM medium at 4°C and transferred every 2 weeks (8).

Measurement of Dry Cell Weight, Lactose Content and Alcohol Concentration

To measure dry cell weights, cells were harvested by centrifugation at 3,500 rpm, dried and weighed. Lactose content was measured by colorimetric method as described previously (Nickerson *et al* (12)). Alcohol concentration was determined by gas chromatography (Hewlett Packard 5890 series II, U.S.A.). The operating conditions were as follows; detector was the thermal conductivity detector (TCD), column material was Porapak Q 80~100 mesh, oven temperature was 150°C, TCD temperature was 200°C, injection temperature was 170°C, carrier gas was helium (flow rate 22 ml/min), and current was 150 mA.

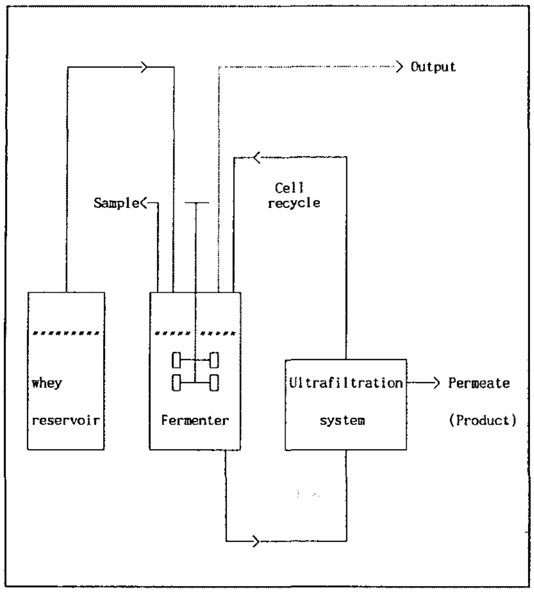

Fig. 1. Schematic diagram of the pseudocontinuous fermentation process and continuous fermentation process of whey. Continuous fermentation process combines an output route and pseudocontinuous fermentation process does not combine an output route.

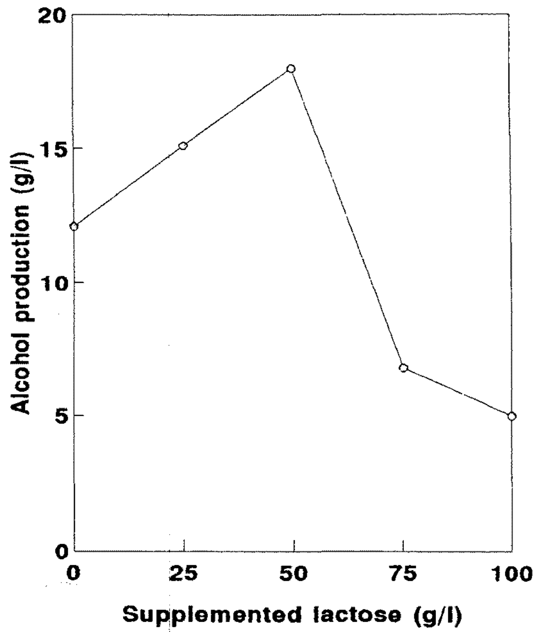

Fig. 2. The alcohol production of *K. fragilis* ATCC 46537 with different amounts of exogenously added lactose to whey (at 28 hours).

Ultrafiltration System (UF)

The UF was conducted at 30°C with a UF cassette (DDS-Division Co. Ltd, Denmark, Mini-Lab 10 system). The molecular cut-off of the filter membrane was 6,000 and the filter area was 0.0336 m^2/4 pieces.

Batch, Pseudocontinuous and Continous Fermentation of Whey

Batch fermentation was operated at 30°C, 200 rpm with an inoculum size of 3% (v/v). The initial pH 4.5 was maintained by the use of citric acid. In case of the pseudocontinuous fermentation, we combined UF system to the conditions set for the batch fermentation process (Fig. 1). Dilution rate was 0.17 hr^{-1} after 28 hours, and it was adjusted to 0.24 hr^{-1} after 66 hours.

Also, for the continous fermentation process, we combined UF system to the conditions set for the batch fermentation process. It was performed with a dilution rate of 0.23 hr^{-1} after 28 hours. The continuous fermentation scheme is dipicted in Fig. 1. The output route for the pseudocontinuous fermentation process is not included.

RESULTS AND DISCUSSION

Searching for an Efficient Alcohol Fermenting Strain in Whey

K. fragilis has been employed in numerous research projects because of its ability to grow rapidly on whey with high yields of alcohol and apparent lack of toxin production (18, 19). When we compared the three strains of alcohol fermenting yeast, alcohol production, dry cell weight and lactose consumption were higher with *K. fragilis* ATCC 46537 than with *S. cerevisiae* IFO 2346 or *K. fragilis* ATCC 28244 (data not shown). Therefore, *K. fragilis* ATCC 46537 was used in this study.

Optimum Conditions for Alcohol Production by *K. fragilis* ATCC 46537 from Whey

The effects of temperature and pH on the production of alcohol by *K. fragilis* from whey were examined under various conditions. We compared the alcohol productions at three varying temperatures (25°C, 30°C and 35°C). The highest alcohol production was obtained at 30°C. When the effect of pH was examined, the alcohol production was 9.0 *g/l* and 9.8 *g/l* at pH 4.0 and pH 5.0, respectively. But, the highest alcohol production, 11.0 *g/l*, was obtained at pH 4.5.

To determine the optimum quantity of lactose supplement, we added 0, 25, 50, 75 and 100 *g/l* of lactose to whey. As shown in Fig. 2, the alcohol production increased continuously until the amount reached 50 *g/l*. However, as the amount was increased further, the alcohol production decreased markedly. This inhibitory effect is likely to be due to the substrate inhibition, for example, dehydration of the cells by high osmolarity. Therefore, the optimum amount of lactose to be added was determined to be 50 *g/l*.

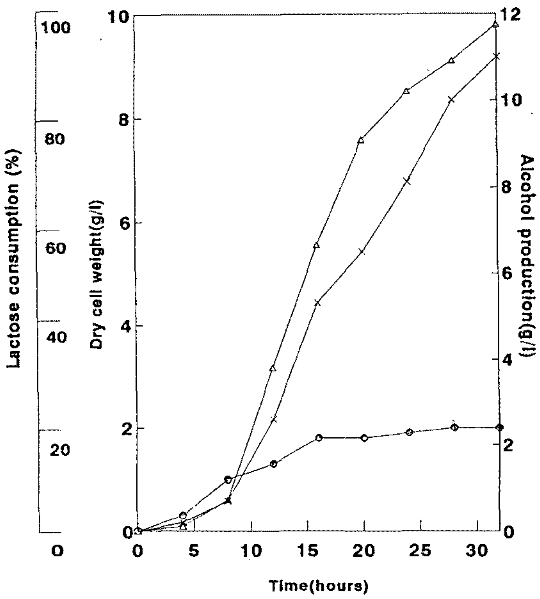

Fig. 3. Kinetics of cell growth, alcohol production and consumption in batch fermentation.
-○-, dry cell weight; ×, alcohol production; -△-, lactose consumption.

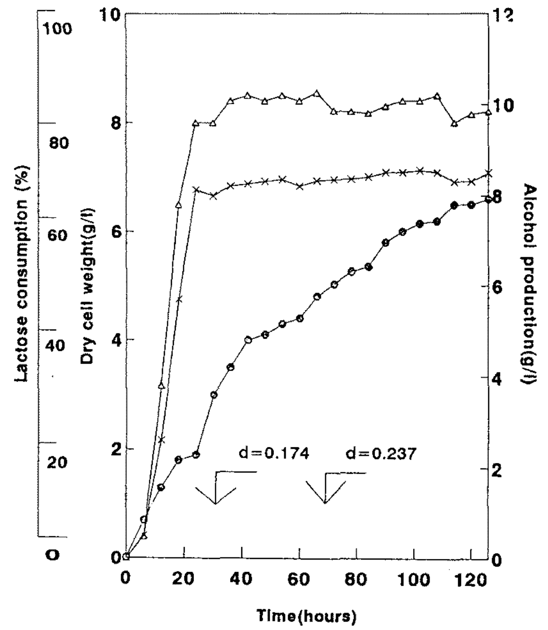

Fig. 4. Kinetics of cell growth, alcohol production and lactose consumption in pseudocontinuous fermentation.
-○-, dry cell weight; ×, alcohol production; -△-, lactose consumption.

Comparison of Batch, Pseudocontinuous and Continuous Fermentation without Lactose Supplementation

Alcoholic beverage can be made from whey under the proper processing conditions, although fresh whey does not contain a sufficient amount of lactose. We initially examined batch fermentation to determine the kinetic pattern of alcohol production. As shown in Fig. 3, after 30 hours of fermentation period, the final results showed that the alcohol production was 11.0 g/*l*, dry cell weight 2.0 g/*l* and lactose consumption 98%. After 16 hours of fermentation (i.e. during the stationary phase), lactose consumption and alcohol production increased continuously, while the dry cell weight decreased. The specific growth rate for the batch fermentation without lactose supplementation was calculated to be 0.2 hr^{-1}.

Janssens *et al.* (8) have previously reported that the UF system could be used for the manufacture of whey protein and alcohol from whey. In regard to this theory, therefore, we performed pseudocontinuous fermentation with cell recycling using the UF system. As shown in Fig. 4, the dilution rate had to be increased gradually because the alcohol production decreased and the cell biomass accumulated increasingly after 28 hours of fermentation. So, UF system was operated with a dilution rate of 0.17 hr^{-1} after 28 hours and 0.24 hr^{-1} after 66 hours. Meanwhile, the batch operation was used to allow the biomass build-up. By 126 hours of fermentation, dry cell weight accumulated to 6.6 g/*l*, while lactose consumption was 79~80% and alcohol production was 7.9~8.5 g/*l* (Fig. 4).

The pseudocontinuous fermentation with UF system could produce alcohol continuously, however, we also examined continuous fermentation to find a process that would operate without increasing the dilution rate during the fermentation. Based on the results from batch and pseudocontinuous fermentation, continuous fermentation was operated with a dilution rate of 0.23 hr^{-1}. As shown in Fig. 5, dry cell weight was 3.0~3.7 g/*l*, lactose consumption was 83~87%, and alcohol production was 8.1~8.7 g/*l*. Maximum alcohol production by pseudocontinuous and continuous fermentation was 8.7 g/*l*. These results clearly demonstrate that the continuous process and whey protein recovery can be easily performed by UF system.

Comparison of Batch and Continuous Fermentation with Lactose Supplementation

As whey naturally contains insufficient amount of lactose, we wondered whether the presence of exogenous lactose would help to increase the alcohol production. According to the report by Mahmoud et *al.* (10) which demonstrated that the high mineral content in whey inhibited the production of biomass and alcohol, we added lactose directly to whey, although the whey lactose could be concentrated with U.F. system.

Maximum dry cell weight, alcohol production and lactose consumption were obtained when 50 g/*l* lactose was added (Fig. 6).

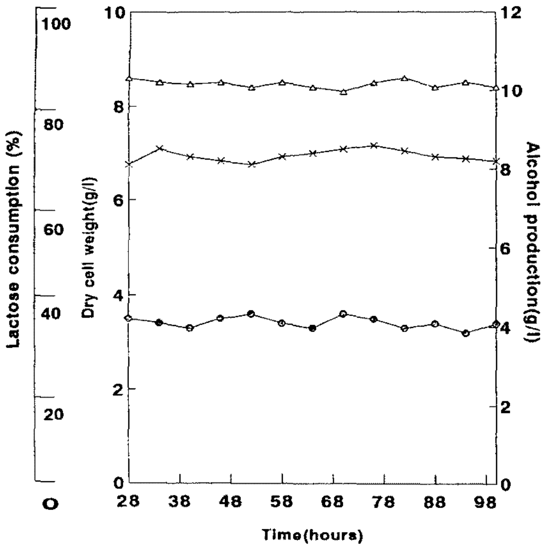

Fig. 5. Kinetics of cell growth, alcohol production and lactose consumption in continuous fermentation.
-○-, dry cell weight; ✳, alcohol production; -△-, lactose consumption.

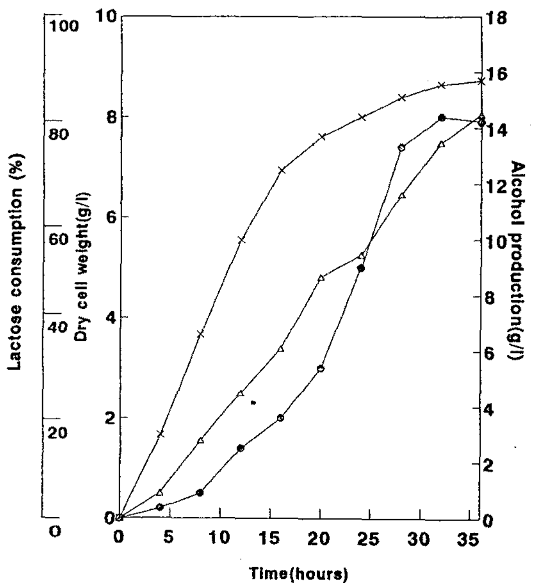

Fig. 6. Kinetics cell growth, alcohol production and lactose consumption in batch fermentation (inoculum 3%, addition of lactose 50 g/*l*).
-○-, dry cell weight; ✳, alcohol production; -△-, lactose consumption.

To compare the kinetic patterns of alcohol fermentation in lactose supplemented whey with that of nonsupplemented one, we performed batch fermentation in lactose supplemented whey. As shown in Fig. 6, dry cell weight was 8.0 g/*l* at 32 hours. Lactose consumption was 80.5% and alcohol production was 15.7 g/*l* at 36 hours. These results indicate that a more rapid growth pattern is obtained in lactose supplemented fermentation than in nonsupplemented one. The culture reached

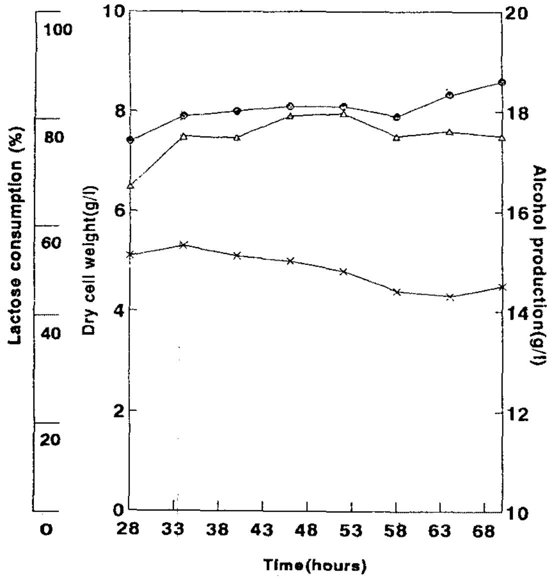

Fig. 7. Kinetics of cell growth, alcohol production and lactose consumption by continuous fermentation (dilution rate 0.485 hr^{-1}, addition of lactose 50 g/*l*).
-○-, dry cell weight; ✳, alcohol production; -△-, lactose consumption.

its stationary phase after 24~28 hours. The specific growth rate was calculated to be 0.49 hr^{-1}, which was higher than the value obtained in batch fermentation without lactose supplementation.

For continuous fermentation, the UF system had to be combined as shown in Fig. 1 with a dilution rate of 0.49 hr^{-1} after 28 hours. This value was higher in lactose supplemented fermentation than in nonsupplemented one. However, to allow the biomass build-up, batch operation was initially used. As shown in Fig. 7, dry cell weight was 7.4~8.6 g/*l* and lactose consumption was 64~79%. Alcohol production was 14.5~15.7 g/*l*, which is significantly higher than the previous reports of 7.1 g/*l* (8). This marked increase in alcohol production is likely to be due to the fact that the initial pH was controlled with citric acid and lactose was added directly to whey, whereas Janssen *et al.* (8) used HCl to control the initial pH and they used whey ultrafiltrate to increase the lactose content in whey.

In conclusion, we have developed an efficient cell recycle system for the continuous alcohol production from whey. Further studies are underway to improve the alcohol yield and flavor of the resulting whey beverage.

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