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Construction of a fuzzy control system for a bioreactor using biomass support particles

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Abstract

A fuzzy control system was applied to a bioreactor in which the flocculent yeast *Saccharomyces diastaticus* ATCC 60715 was immobilized by entrapment in a reticulated polyurethane foam cube (6 mm × 6 mm × 6 mm, biomass support particle (BSP)). The fuzzy inference algorithm was used to calculate the suitable level of glucose feed rate from the monitored data of the CO_2 evolution rate (CER) and the integrated amount of CO_2 evolution. One of the main concerns is the mass transfer of glucose in the BSP in which *S. diastaticus* was immobilized. With the BSPs, glucose diffusivity becomes lower and starvation of the cells can occur if the control system is not quick enough to respond to the starvation. The fuzzy control system was successful in controlling the glucose feed pump to maintain a suitable glucose concentration for the smooth continuation of ethanol production.

Keywords: Biomass support particle; Bioreactor; Fuzzy control; Mass transfer; Membership function

1. Introduction

Recently, the passive immobilization technique using porous biomass support particles (BSPs) originally developed by Atkinson et al. [1] has been applied to a wide variety of microbial, animal, and plant cell systems. In particular, bioreactor systems with yeast immobilized by BSPs have been actively investigated [2–5].

In the case of ethanol production by immobilized yeast, the glucose consumption rate in the yeast support particles is high, and the glucose concentration in the particles is low compared with the concentration outside the particles. Therefore, we must estimate the critical concentration outside the particles, which means that if the outside glucose concentration is below the critical level, starvation will occur inside the particle. Therefore, we must keep the glucose concentration higher than the critical level in order to avoid the depletion of sugar inside the particle. For the determination of the critical level, we must consider glucose transfer from the outside of the particles to the immobilized cells inside the particles by means of the diffusion of glucose. The simulation algorithm for the bioreactor with the immobilized system [6–8] was applied for the estimation of the critical concentration outside the particle. However, the BSP is made of urethane foam, which is a very soft material, and

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the mass transfer might be affected by the deformation due to the mechanical stress. Furthermore, cell growth occurs inside the BSP, and the thickness of the dense cell layer changes, which can also affect the mass transfer of glucose. Therefore, it is difficult to estimate the critical concentration accurately, and we must use other variables for the detection of starvation inside the BSPs. We adopted the CO_2 evolution rate (CER) as that variable, because it is approximately proportional to the ethanol production rate and sensitively reflects the metabolic activity inside the cell body. However, CER can reflect factors other than the depletion of glucose, e.g. the depletion of other components, and the pH change by the addition of a pH control reagent (NaOH, HCl). Therefore, we cannot detect the beginning of starvation precisely from the amount of CO_2 evolution or CER independently.

However, we can imagine that the possibility of starvation is very high if the CER dropped remarkably and the glucose concentration estimated by the mass transfer simulation using the amount of CO_2 evolved is lower than the critical level calculated roughly by the mass transfer simulation.

A control policy, which can be expressed linguistically, and mainly comes from the experience, and cannot be determined exactly and deterministically, can be used for computer control based on the fuzzy theory [9].

The fuzzy theory has been applied to various fermentation processes, e.g. glutamic acid production [10,11], Japanese sake brewing [12,13], enzyme production [14,15], enzyme inhibitor production [16], and Vitamin B_2 production [17]. However, we could not find any report on the application of the fuzzy control to bioreactor system with immobilized enzymes and/or cells. We studied the efficiency of the application of fuzzy control in a bioreactor system with immobilized cells to avoid starvation inside the BSP carrying *Saccharomyces diastaticus* or the inactivation of alcohol production.

2. Experimental

2.1. Strain and media

The strain used of this research was the flocculent yeast *S. diastaticus* ATCC 60715. For the preculture, we used five flasks, each containing 100 ml of preculture medium containing 10 g/l yeast extract, 20 g/l polypeptone, and 30 g/l glucose. One hundred pieces of BSPs were inoculated into each flask with one to two loops of the yeast strain. They were incubated at 30 °C for 1–2 days on a reciprocal shaker.

The BSP is reticulated polyurethane foam cubes (6 mm × 6 mm × 6 mm).

We carried out repeated fed-batch fermentation as main cultures, and used the following three culture media. One is the initial medium that contains 10 g/l yeast extract, 20 g/l polypeptone and 50 g/l glucose. Another is the feed medium containing 130 g-glucose/600 ml, and the other is the replacement medium containing 50 g-glucose/l.

2.2. Main culture

The bioreactor used is the airlift 2 l jar fermentor (Model BMJ-02PI, ABLE, Tokyo). Initial working volume is 0.6 l. The complete hardware configuration, including the control system, is shown in Fig. 1. One hundred pieces of the BSPs carrying *S. diastaticus* are harvested aseptically from the precultures and transferred to the fermentor containing the initial medium. The temperature and the pH were kept constant at 30 °C and 6.0, respectively. When the glucose concentration of the medium decreased to the critical level, the feed pump began supplying the feed medium to the fermentor. When the feed medium for the batch was completely used and the glucose concentration reached the critical level, the fermentation medium was changed to the fresh replacement medium, and the next batch was started. When the glucose concentration of the medium decreased to the critical level, the feed pump automatically started again. The cycle was repeated until three to six batches of replacement medium were used.

2.3. Analytical methods

A volume of 10 ml of culture broth were taken as samples at appropriate time intervals during cultivation. The samples were centrifuged at 4 °C for 5 min at 15,000 rpm, and the supernatants were used for the measurement of glucose and ethanol concentrations.

The glucose concentration is analyzed using the Yellow Springs Instrument, Model 2700/115 V Dual

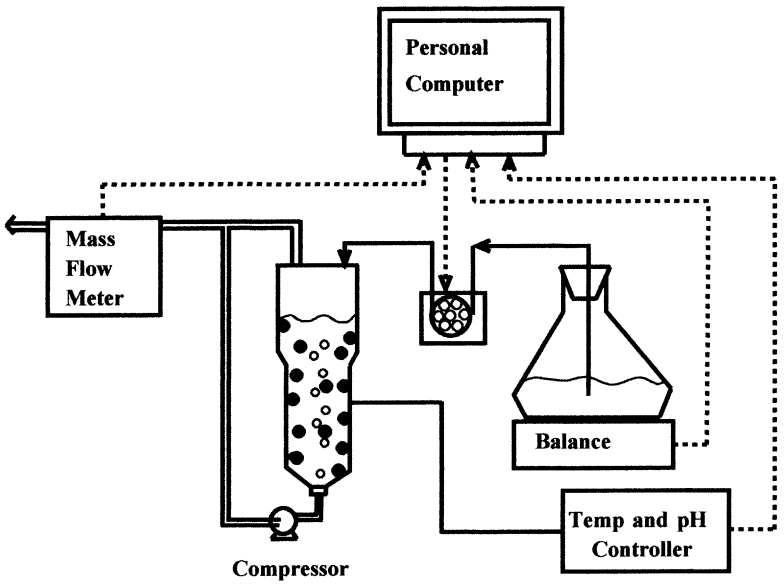

Fig. 1. Intelligent bioreactor system.

Channel Analyzer. The ethanol concentration is analyzed using the Hitachi G-3000 gas chromatograph with the high resolution capillary column TC-1 (30 m × 0.53 mm i.d., GL Science Co. Ltd., Tokyo).

2.4. On-line estimation of sugar concentration

The CER from the fermentor was detected by the mass flow meter (Model 3850 MS-G2, KOFLOC Co. Ltd., Tokyo), because gas other than CO_2 cannot be generated in the alcohol production system. This signal was transferred to the control unit attached to the fermentor. Data from the control unit (temperature, pH, DO, and CER) were sent to the computer, which displayed the present culture state and the time courses of the state variables. The amount of glucose that was fed into the fermentor was also monitored using a balance, which was connected to the computer. The software for monitoring and controlling the hardware was developed in Basic language, and the program for fuzzy inference was developed in Fortran language to shorten the time consumed. The monitoring data could be stored in the form of a data sheet in Excel. A schematic view of the software configuration is shown in Fig. 2.

In the first batch of fermentation, the cell utilizes glucose for both growth and ethanol production. From the subsequent batches, the glucose is utilized mainly for ethanol production. The fermentation system is closed except for the outlet through the mass flow meter that detects the outgoing CO_2 as can be seen in Fig. 3. The amount of glucose converted to ethanol is proportional to the amount of CO_2 generated, which was estimated by the integration of the CER. Therefore, we can perform the on-line estimation of the amount of glucose consumption using the proportion and the amount of CO_2 generated. The glucose content in the culture solution can be calculated using the following equation.

$$\text{Glucose content} = \text{initial glucose} - \text{estimated glucose consumption} + \text{glucose fed}$$

2.5. Software development for fuzzy control

The glucose concentration inside BSPs is lower than that outside BSPs, because the cells immobilized in BSPs consume the glucose in the BSPs. The distribution of the glucose concentration cannot be measured

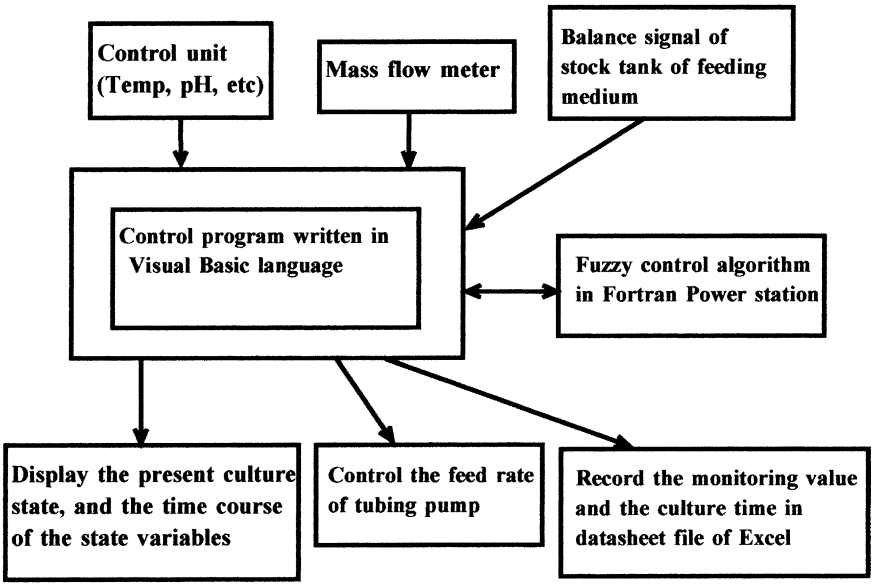

Fig. 2. Software configuration.

easily, but it can be estimated by means of the simulation based on the mass transfer mechanism inside the BSP following the reported simulation [6–8].

As a result of the simulation, we roughly estimated the critical glucose concentration to be 25 g/l, that is, starvation will occur if the glucose concentration outside BSPs is lower than 25 g/l. However, we cannot detect starvation from only the estimated glucose concentration sensitively because BSP is a very soft material, i.e. urethane foam, and its shape can be changed by mechanical stress. Furthermore, cell growth occurs inside the BSPs, which also affects the mass transfer of glucose and the glucose consumption rate.

Based on experience concerning the starvation state in culture experiments, we found that the CER, which reflects the amount of glucose consumed, decreases when the starvation occurs in the bioreactor. Therefore, we adopted two variables, i.e. CER and the amount of CO_2 equivalent to the glucose content in the bioreactor (EGC).

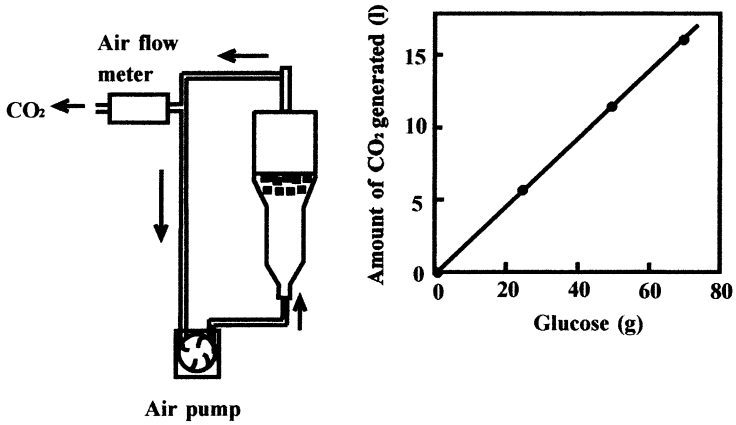

Fig. 3. On-line glucose estimation from the amount of CO_2 generated.

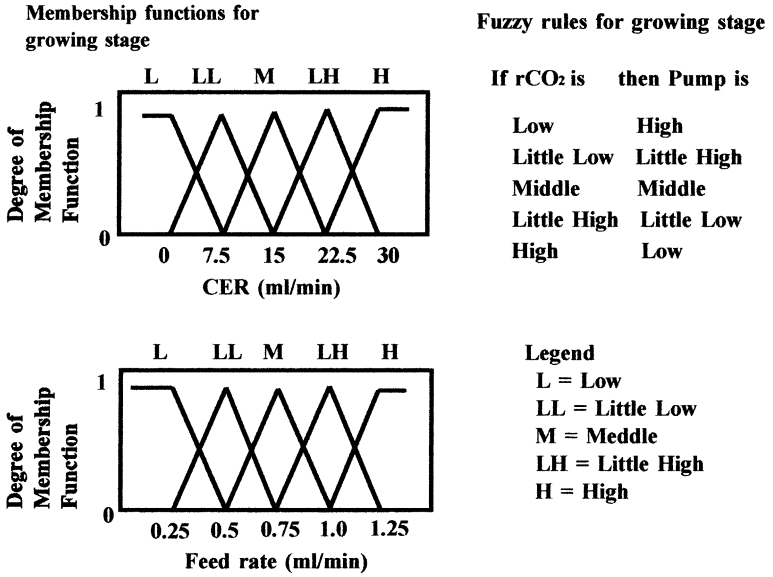

Fig. 4. Fuzzy sets and fuzzy rules for growing stage.

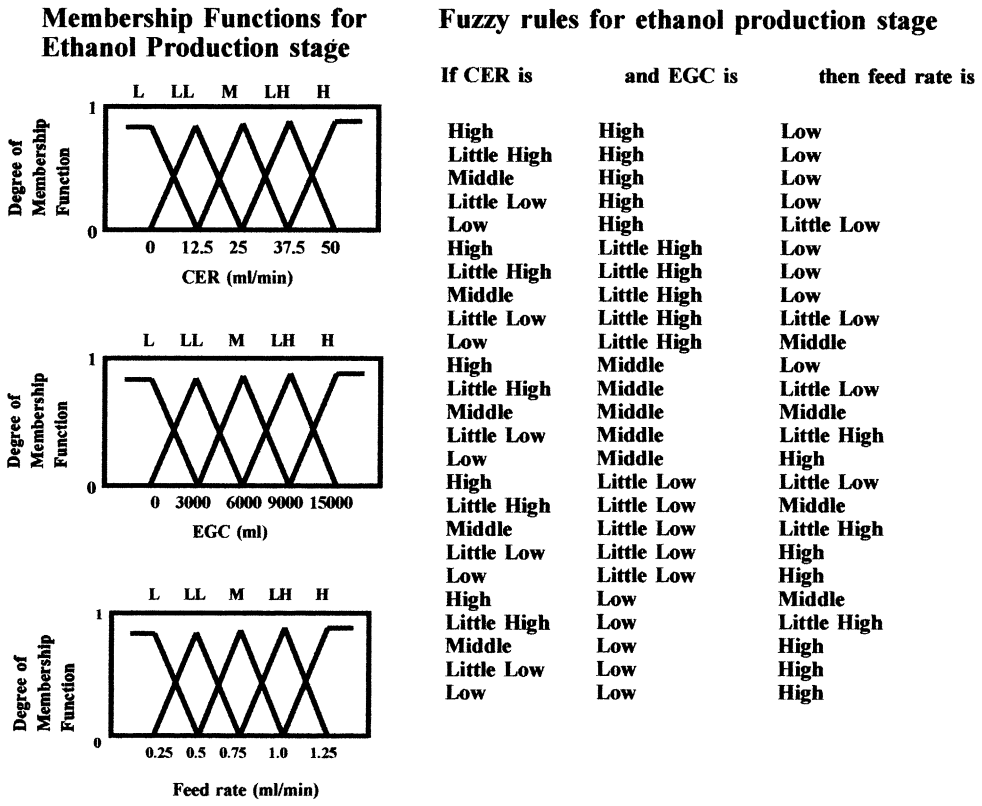

Fig. 5. Fuzzy sets and fuzzy rules for ethanol production.

After gathering data from culture experiments, the state variables in the antecedents (CER and EGC) and the operative variable (glucose feed rate) in the consequent in production rules were grouped into different labels such as low, little low, middle, little high and high. These groups can overlap each other so that at any point, there is no abrupt change from one label to another. The membership functions are shown in Figs. 4 and 5.

The production rules were also defined as follows. In the first batch, some of the glucose was consumed for cell growth, and the relationship between CO_2 generation and glucose consumption shown in Fig. 3 could not be applied to the first batch. Therefore, we used only the CER as the variable in the antecedent in fuzzy rules, as shown in Fig. 4. From the next batch, glucose was converted mainly to ethanol, and the glucose feeding policy could be determined from the CER and EGC by fuzzy inference. The membership functions and fuzzy rules from the second batch are shown in Fig. 5.

3. Results and discussion

The developed software was tested in a culture experiment using the experimental system shown in Fig. 1. One example of time courses of the culture experiment is shown in Fig. 6. Glucose concentration could be kept above 25 g/l, and ethanol production continued until the fifth batch under severe conditions, that is, the medium contained only glucose was fed into the reactor from the second batch, which means that ethanol production could not be continued if starvation occurred. Second batch to sixth batch were started at 34, 48, 55, 68, and 88 h, respectively, and the culture solutions were changed to the fresh replacement media just before those culture times, which were shown as sudden increase of glucose concentration by the replacement operation in Fig. 6. The average yield was 0.38 g-ethanol/g-glucose. In the fifth batch, the decrease of the ethanol production rate was observed from the extended time for the increase of ethanol concentration up to 37 g/l and the decrease of CER (data not shown). The decrease is supposed to come from the decrease of the cell activity for ethanol production because the glucose and ethanol concentrations were kept in a moderate level for alcohol production throughout the batch. We used the initial medium as a replacement medium for the sixth batch, resulting in the complete recovery of the production rate. The time course of the first batch showed that the high glucose concentration inhibits cell growth. Therefore, we carried out another culture experiment in which the initial glucose concentration in each batch was lowered to 25 g/l. The total amounts of glucose added in the repeated fed-batch cultures shown in Figs. 6 and 7 are 550 and 250 g, respectively. As shown in Fig. 7, the time of the first batch was shortened compared with the previous culture experiment, and the average yield increased to 0.45 g-ethanol/g-glucose. These results indicate that fuzzy control can be effective for the smooth production of ethanol by the immobilized cell system.

Generally it is difficult to measure the substrate concentration and/or product concentration inside the support particles with immobilized cells, and the starvation of the substrate or excess accumulation of product can damage the ethanol production in the bioreactor. The simulation cannot predict the exact

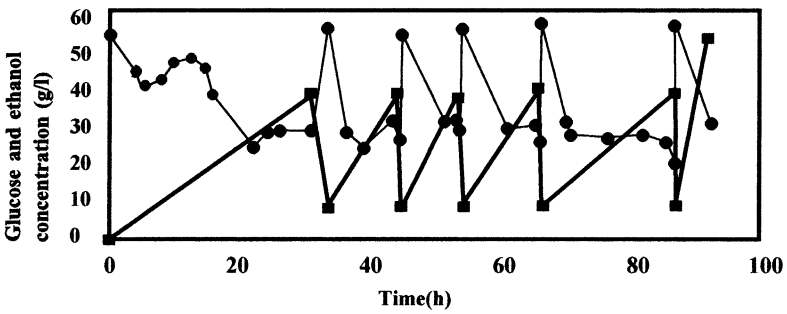

Fig. 6. Time courses of glucose and ethanol concentrations in repeated fed-batch culture: (●) glucose, (■) ethanol.

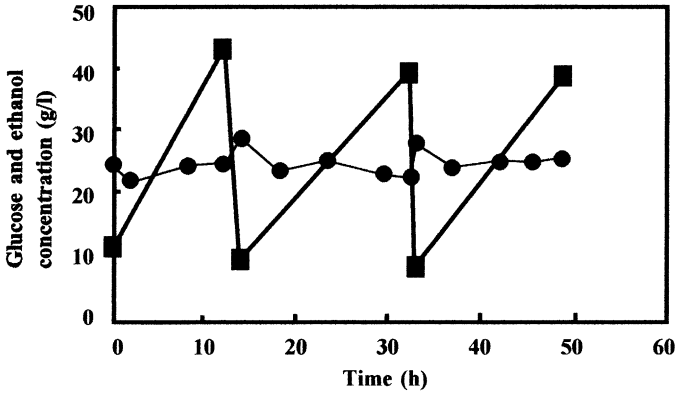

Fig. 7. Ethanol production by repeated fed-batch culture under low glucose concentration: (●) glucose, (■) ethanol.

state inside the BSP because of factors such as the cell growth, deformation of the BSP, and presence of turbulence or small eddies inside the BSP. Therefore, fuzzy control such as the one in the present research is quite effective for the control of a bioreactor with immobilized cells.

Acknowledgements

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