Journal of Biotechnology, 24 (1992) 75–83

BIOTEC 00754

Fuzzy reasoning system for fault diagnosis of physiological activities in a cultivating process

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(Received 28 January 1991; revision accepted 17 August 1991)

Summary

Aiming at development of a system which supports cultivating operations, a method to diagnose physiological activities in a cultivating process is presented, and a fuzzy expert system for diagnosing *Lactobacillus casei* cultivating process is implemented in this paper. This system can calculate specific rates of cell growth, substrate consumption, and product formation with measuring cell mass concentration, substrate concentration, and product concentration by using a turbidity sensor and HPLC. A database is implemented, where standard curves on specific rates representing characteristics of microorganisms are stored according to normalized substrate consumption. Comparing the calculated specific rates with standard values derived from the database, the system diagnoses physiological activities of the microorganisms. As a case study, a knowledge base for diagnosing lactic acid production process is implemented. The use of fault diagnosis on pH malfunctions by the expert system proves its reasonable performance.

Expert system; Fuzzy reasoning; Fault diagnosis; Physiological activity; Lactic acid production; Database

Introduction

Industrial scale of cultivation plants are moving towards automation. However, the lack of on-line sensors for measuring important variables like cell mass,

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substrate, or product concentration, and various difficulties to establish mathematical models for the complicated and diverse cultivating processes hinder developments in the bioprocess automation. Therefore, the bioprocesses are still operated by expert operators who stock their knowledges by empirical and/or heuristic decisions. An automatic monitoring and controlling system (BIOACS: BIO Advanced Control System) was developed (Endo et al., 1989), which is capable of monitoring state variables on-line and controlling the process optimally on the basis of physiological data of microorganisms. By using this BIOACS, an expert system has been reported for diagnosing mechanical faults of the cultivation system and misoperations by operators (Asama et al., 1990). The objective of this work is to improve the function of the expert system so that it can diagnose the faults in terms of physiological activities of a microorganism during its cultivating process. The lactic acid batch cultivating process was adopted as a case of study here. Concerning lactic acid cultivation, though an on-line monitoring system for process automation was reported (Nielsen et al., 1989), utilization of the monitored variables for intelligent control or diagnosis was not explicitly discussed. In the method discussed in this paper, the specific rates of cellular growth, substrate consumption and product formation were chosen as parameters representing the physiological activities of the microorganism, and cultivation state can be diagnosed with the calculation of specific rates related to measured state variables.

Materials and Methods

Microorganism. Lactobacillus casei sp.

Precultivation media. Rogosa media: Composition of Rogosa media is shown in Table 1. The pH of precultivation media should be 6.8. Composition of salt solution in Table 1 is shown in Table 2.

TABLE 1

Rogasa media

Trypticase peptone	1.0%
Yeast extract	0.5%
Tryptose	0.3%
K_2HPO_4	0.1%
KH_2PO_4	0.1%
Diammonium hydrogen citrate	0.2%
Tween 80	1.0 ml l^{-1}
Sodium acetate trihydrate	0.17%
L-Cysteine monohydrochloride	0.02%
Dextrose	2.0%
Agar	1.5%
Salt solution	5.0 ml l^{-1}

TABLE 2

Salt solution in Rogasa media

$MgSO_4 \cdot 7H_2O$	11.5%
$FeSO_4 \cdot 7H_2O$	0.68%
$MnSO_4 \cdot XH_2O$	2.4%

TABLE 3

Media

Glucose	2.5%
Corn steep liquor (CSL)	10.0%
K_2HPO_4	0.1%
KH_2PO_4	0.1%
$MnSO_4 \cdot 2H_2O$	0.006%

Cultivation media. Composition is shown in Table 3. The salt solution was made acidic with H_2SO_4 and stored in the cold.

Growth conditions. Temperature was kept at 35°C and pH was controlled at 6.5 by 5 N KOH, agitation speed was maintained at 150 rpm.

Equipment and analysis methods

Cultivations were done by using minijar-type bioreactors with 2.0 l and a jar-type bioreactor with 15.0 l working volume. Minijar-type bioreactors were mainly used for investigation of process characteristics including specific rates in standard cultivating conditions. Then the characteristics were verified also in large scale experiments using the jar-type bioreactor which is connected to the BIOACS. This system makes it possible to measure on-line the state variables like cell mass, substrate and product concentration. The cell mass concentration is measured using a turbidity sensor, and both substrate and product concentration are analyzed by using an on-line sampling unit and liquid chromatograph. All the data are collected and fed to the process computer. The computer calculates the specific rates of cellular growth, substrate consumption and product formation immediately via Kalman filter (Endo and Nagamune, 1983) which can eliminate the noises.

Cultivation kinetics

The case of study is an anaerobic batch culture cultivation of lactic acid production. Fig. 1 shows the time course behavior of cell mass, substrate and product concentration under normal cultivation conditions. The lag phase is about 5–7 h and is followed by a short exponential cell mass growth. The cell growth is

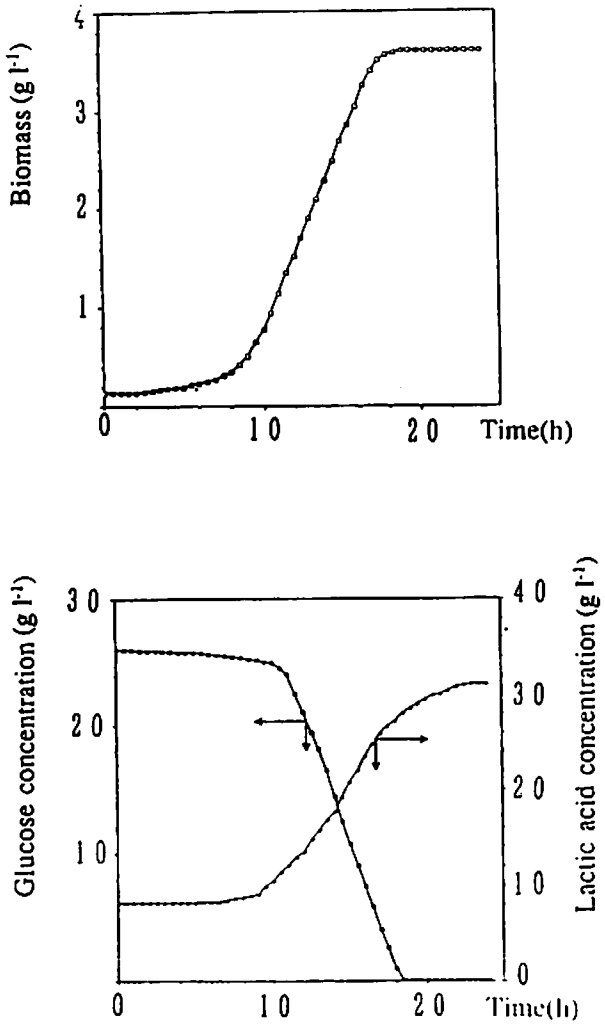

Fig. 1. Time course behavior of cell mass, substrate and product concentration (g l^{-1}) under normal conditions.

mainly linear. The stationary phase is reached in about 16–18 h. The lactic acid production is mainly associated with growth, some production is non-growth associated and product concentration reached the maximum point within 24 h. Product inhibition usually controls the production of lactic acid. Cells died with concentration of 20 g l^{-1} of lactic acid or pH under 4.0.

Database implementation

The specific rates of cell growth (μ), substrate consumption (ν), and product formation (π) were calculated as parameters which represent physiological activities

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{1}$$

$$\nu = -\frac{1}{X}\frac{dS}{dt} \tag{2}$$

$$\pi = \frac{1}{X}\frac{dP}{dt} \tag{3}$$

In the equations above, X, S and P denote the cell mass, substrate and product concentration respectively and t denotes the cultivation time. A database representing the standard curves of each normalized specific rate of cellular growth (μ^*), substrate consumption (ν^*) and product formation (π^*) against substrate normalized (S^*) was calculated using the data of several cultivations under normal conditions and stored in BIOACS. In the following equations μ_{max}, ν_{max} and π_{max} represent the maximum values of the cell mass growth, substrate consumption and product formation respectively, and S_0 denotes the initial substrate concentration.

$$\mu^* = \frac{\mu}{\mu_{max}} \tag{4}$$

$$\nu^* = \frac{\nu}{\nu_{max}} \tag{5}$$

$$\pi^* = \frac{\pi}{\pi_{max}} \tag{6}$$

$$S^* = \frac{S}{S_{max}} \tag{7}$$

In the standard curves shown in Fig. 2, it is possible to observe the different phases of the cultivation kinetics, and they were named as exponential, first

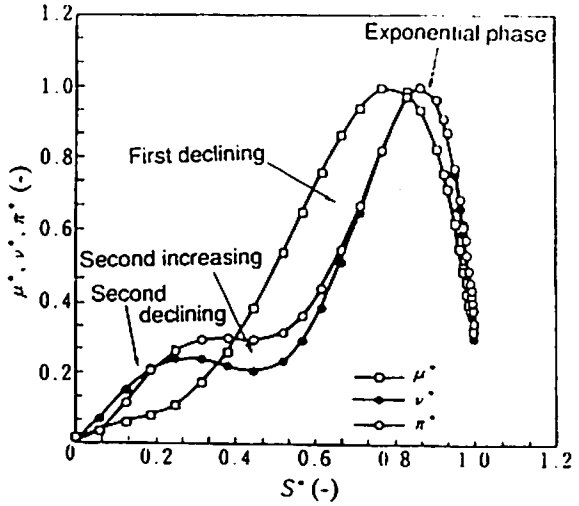

Fig. 2. Standard curves of specific rates and kinetic phases. These curves were obtained under normal cultivation conditions. $\mu = (h^{-1})$, ν = glucose per cell per time (g g^{-1} h^{-1}), π = lactic acid per cell per time (g g^{-1} h^{-1}).

declining, second increasing and second declining phases. Under normal conditions, the behavior of the phases in the standard curves can be observed.

Detection of faults in terms of physiological activities

The expert system receives not only the on-line filtrated values of the actual specific rates of cell mass growth, substrate consumption and product formation, but also the standard values of specific rates calculated from database. If the difference between the values is within a certain threshold, the cultivation is regarded as normal, but if not the system starts the diagnosis. The expert system also compares the cultivation kinetics phases getting additional information. It also indicates some explanations of the results and some remarks about checking items to verify device functions. Under monitoring state, faults are investigated every 20 min. The expert system was developed in object oriented language Smalltalk/V in a Macintosh II computer with 5 MB memory and 102 MB hard disk, and was implemented using an expert shell developed by Aarts et al. (1990).

Malfunction diagnosis

The knowledge base for malfunction diagnosis is represented by a network of objects. Malfunctional events are described by natural language such as 'μ is low' and are defined by their characteristics such as setpoints and tolerances. The process variables can be represented by fuzzy sets. Fig. 3 shows a trapezoidal membership function of the fuzzy variable 'pH is low'. The system contains five fuzzy classes of 'very high', 'high', 'low' and 'very low'. Knowledge for diagnosis is a network of event nodes and links between nodes. Relations between events which represent causality of faults are defined as links with certainty factors. Fig. 4 shows a part of the knowledge network. Several kinds of nodes can be defined. Start nodes represent classes of the process variables which correspond to root causes of malfunctions. End nodes represent malfunctional events which are

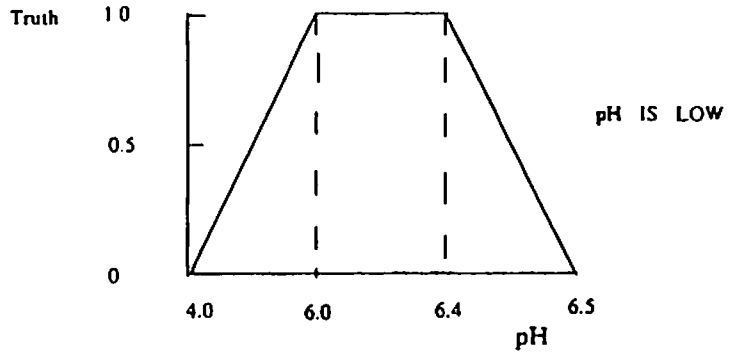

Fig. 3. Membership function of the fuzzy variable 'pH is low'.

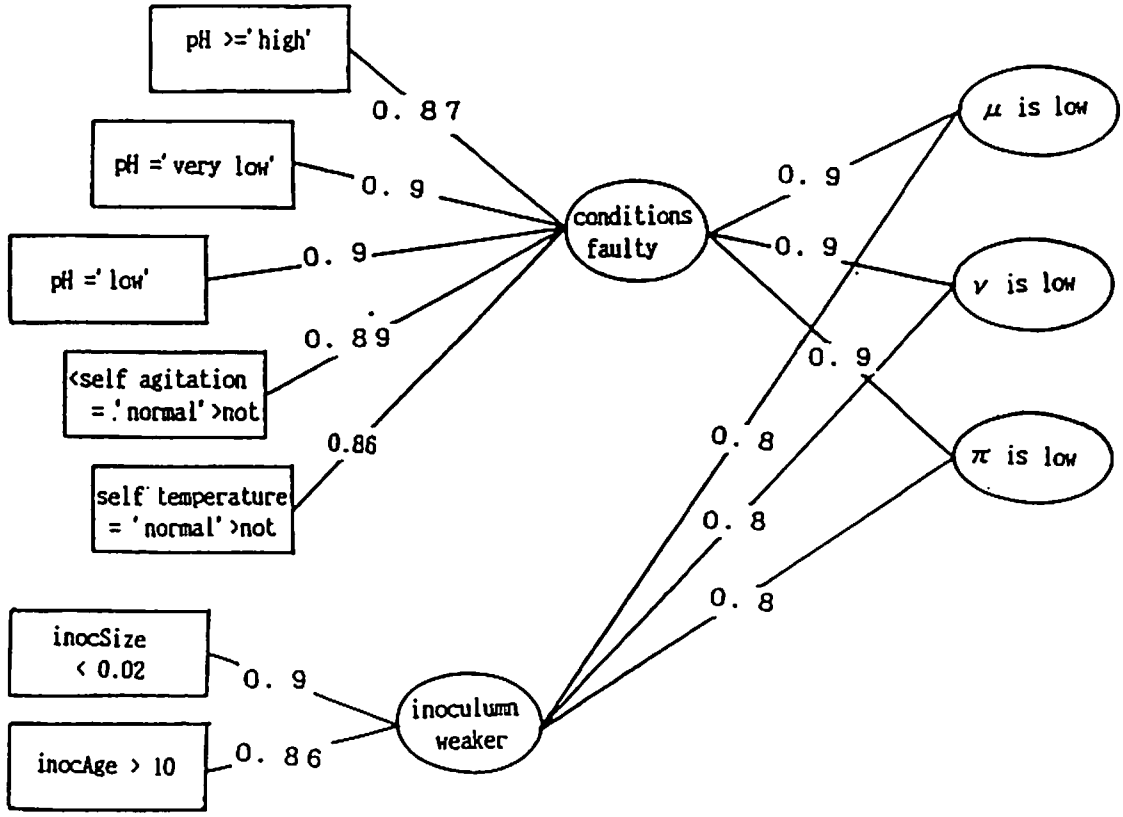

Fig. 4. A part of the knowledge base network for fault diagnosis.

classes of specific rates in this case. Start nodes and end nodes are connected using 'or' and/or 'and' nodes. When the fault is observed, the system refers to the network firstly from the collection of the end nodes and traces whole chain from end to start node and calculates the truth factor value. The truth factor value can be calculated by multiplying the value of each node with the connection value. The fault reported after diagnosis is the one possible candidate of root causes with the highest truth factor value. The system displays the faults and causes as a sequence of probabilities. Fuzzy reasoning is used in searching the network. In the network the knowledge is divided to specific knowledge of microbe, general knowledge of microbial activities, knowledge of each specific rate and new knowledge obtained during the cultivation.

Cultivation results under different pH conditions

Experiments under different pH conditions were executed and their effects on specific rates were investigated by comparing with standard specific rates. Fig. 5 shows the effect of the pH changes on the μ value. The control value of pH was fixed from the beginning in the case of pH 3.3, 5.0, 8.0 and 9.3. At pH 3.3 and 9.3,

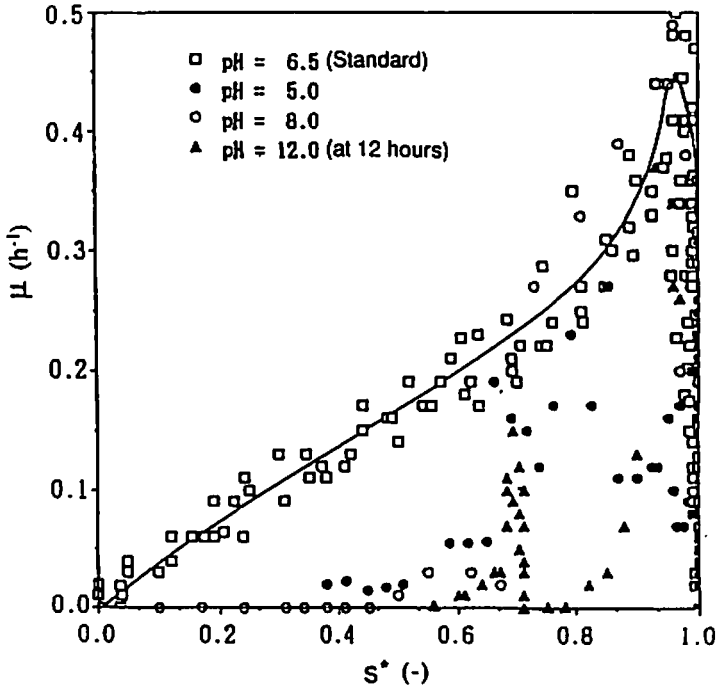

Fig. 5. Effect of pH on the specific cell mass growth rate.

no growth was observed. In the case of pH 12, pH was changed at 12 h for 1 h. When the control value of pH was 5.0 or 8.0, the maximum values of specific rates were lower than the maximum values of specific rates under normal conditions and the values of specific rates were lower during the whole cultivation time. In the case of pH 12 the maximum value of specific rates were equal to the value in the normal conditions and lower values of specific rates could be detected after the change. Small pH changes from 6.5 to 6.9 at 8 h for 3 h of cultivation, when the microbe is in the exponential phase, had very little effect on the specific rate values. But when the microbe was at the lag phase or starting exponential phase, the same pH changes lowered the specific rate values. The developed expert system is capable of diagnosing the faults from pH control.

Conclusions

A method of fault diagnosis in cultivating processes concerning physiological activities based on specific rates is presented. As a case of study, the database on normal conditions and knowledge for fault diagnosis concerning the *Lactobacillus casei* cultivating process was implemented. Effects of pH conditions were clarified by experiments, and the specific rates were proved to be more sensitive to the pH changes than the data of biomass, substrate and product concentration. Using the developed expert system, it was possible to diagnose the faults from pH control. The function of the expert system for fault diagnosis will be improved by more knowledge acquisition.

Acknowledgement

The authors thank Dr. T. Nagamune in RIKEN, who gave valuable advice and discussions on development of the diagnosing method.

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