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Diagnosing lactic acid fermentation based on specific rates of growth, substrate consumption and product formation

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Abstract. The on-line calculated specific rates of growth, substrate consumption and product formation were used to diagnose microbial activities during a lactic acid fermentation. The specific rates were calculated from on-line measured cell mass, and substrate and product concentrations. The specific rates were more sensitive indicators of slight changes in fermentation conditions than such monitored data as cell mass or product concentrations.

List of symbols

μ	1/h	specific rate of cell growth
ν	1/h	specific rate of substrate consumption
π	1/h	specific rate of product formation
μ^*	-	dimensionless specific rate of cell growth
ν^*	-	dimensionless specific rate of substrate consumption
π^*	-	dimensionless specific rate of product formation
μ_{max}	1/h	maximum specific rate of cell growth
ν_{max}	1/h	maximum specific rate of substrate consumption
π_{max}	1/h	maximum specific rate of product formation
X	g/l	cell mass concentration
S	g/l	substrate concentration
S^*	-	dimensionless substrate concentration
S_0	g/l	initial substrate concentration
P	g/l	product concentration

1 Introduction

Microbial physiology during a fermentation process can be evaluated by measuring cell growth, and substrate and product concentrations. Specific rates calculated from these variables further describe the physiology. The conventional off-line determinations are often time consuming, and the results are available only after the completion of the fermentation. Frequent and repeated sampling during off hours when long series of experiments are done also can cause problems.

The Bio Advanced Control System (BIOACS) developed by Endo et al. [1, 2] was used in diagnosing microbial activities on the basis of the on-line calculation of the specific rates of growth, substrate consumption and product formation.

A batch lactic acid fermentation of Lactobacillus casei was selected for a case study. Lactic acid fermentation is interesting since often only pH, temperature and other physical parameters are monitored on-line, and thus the on-line measurements with calculations using the BIOACS system could bring out new valuable information about the process.

2 Materials and methods

2.1 Organism and culture conditions

Lactobacillus casei sp. ATCC 27092 from Yakult (Tokyo, Japan) was used as the micro-organism. The strain was stored on Rogosa medium with 15 g/l of agar added [3] at 4 °C, and precultivated twice in 5 ml of the Rogosa medium for overnight at 35 °C. Rogosa medium was also used as the seed culture medium, and the initial pH was adjusted to 6.8. The seed culture was incubated at 35 °C for 10 to 12 hours depending on the experiment. The inoculum volume was 1% of the total fermentation volume. The fermentation medium consisted of glucose 2.5%, corn steep liquor (CSL) 10%, K_2HPO_4 0.1%, KH_2PO_4 0.1% and $MnSO_4 \times 2H_2O$ 0.006%.

During fermentation the temperature was controlled at 35 °C, and pH at 6.5 by addition of 5 molar KOH. Agitation rate was set to 150 rev/min to keep the pH constant in every part of the fermentor. No aeration was used.

2.2 Fermentation equipment and analyses

Fermentations were carried out both in 3.0 dm^3 fermentors from Iwashiya K. Sawada Co. and a 30 dm^3 fermentor. The working volumes of these fermentors were 2.0 dm^3 and 15 dm^3 respectively. The larger fermentor was connected to the Bio Advanced Control System (BIOACS) [1, 2]. BIOACS makes possible to measure on-line cell mass, and substrate and product concentrations. Cell mass concentration was measured by a turbidity sensor, and the substrate and product concentrations were analyzed by a high performance liquid chromatograph (HPLC) connected to an on-line sam-

pling unit of cell free medium. The turbidity sensor uses laser light (620 nm) scattering and it is sterilizable by installing into the fermentor directly.

The on-line sampling unit was a ceramic filter with a pore size of 0.2 μm, pumps, a flow cell and a controlled syringe. The filter unit was sterilizable and installed into the fermentor. All the measured data were transferred to a process computer with sampling times of one minute for the cell mass, and 20 minutes for substrate and product concentrations. When the 3.0 dm^3 fermentors were used, substrate and product concentrations were analyzed off-line by the chromatograph (HPLC) of the BIOACS system. Cell mass concentrations were also measured off-line with the turbidity sensor of the BIOACS system. The dry cell weight was always determined by drying a 5 ml centrifuged sample to a constant weight at 105 °C.

3 Calculation of specific rates of cell growth, substrate consumption and product formation

.ıe specific rate of growth μ, substrate consumption v and product formation π were calculated on-line by BIOACS using equations (1) to (3):

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

$$v = \frac{-1}{X} \cdot \frac{dS}{dt}, \tag{2}$$

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

where X, S, and P are concentrations of biomass, substrate and product respectively and t is time of operation.

Kalman filter [4] was applied to dampen the noise caused by the measuring equipment, changes in the medium, and other error signals characteristic of bioprocesses. The physiological activities in a batch cultivation are functions of the ·nitial substrate concentration, initial cell mass concentra-
ɔn and substrate concentration in the culture broth [5]. When the dimensionless specific rate (μ^*, v^*, π^*) as given by Eqs. (4) to (6) were plotted as the function of dimensionless substrate concentration S^*, Eqn. (7), characteristic curves such as shown in Fig. 1 were obtained.

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

$$v^* = \frac{v}{v_{max}}, \tag{5}$$

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

$$S^* = \frac{S}{S_0}. \tag{7}$$

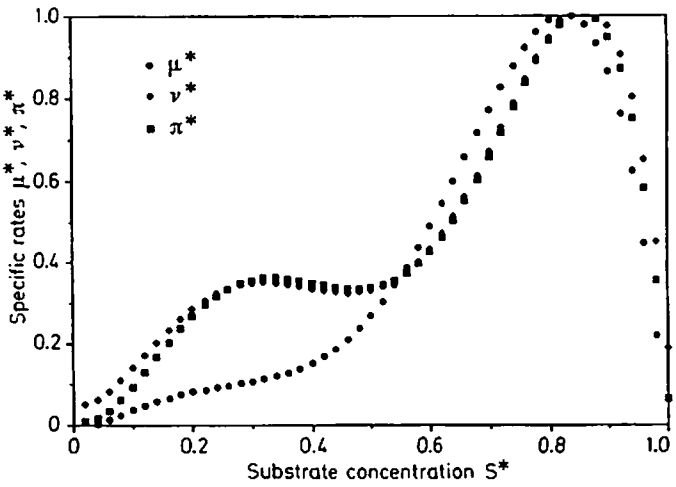

Fig. 1. The specific rates of cell growth (•), substrate consumption (•) and product formation (▪) expressed as a function of substrate concentration

After several experiments were run under the same conditions, a database could be generated from a set of curves shown in Fig. 1, and it can be implemented in the BIOACS. This database could then be used as a set of ideal standard curves for the diagnosing of specific rates. Nagamune et al. [5] have previously used this approach in determining the optimal feed strategy for fed batch fermentations.

During a fermentation process different states of microbial activity were observed based on the shape of the specific rate curves. For the lactic acid fermentation studied as an example, the states were called exponential, first declining, second increasing and second declining phase according to the variations in the specific rate curve (Fig. 2). By determining a phase of the fermentation for each specific rate, additional infomation could be obtained for the fault diagnosis as has been discussed by Halme [6].

4 Diagnosis of microbial activities based on different specific rates

To investigate whether microbial physiology could be diagnosed based on specific rate determinations, a series of cultivations were done at the various pH levels of 3.3, 5.0, 6.5, 8.0 and 9.3. As it was expected the cultivations at pH 3.3 and 9.3 showed no growth. Other cultivations were compared to the standard condition in which pH value was set at 6.5. All the specific rates were lower than those of the standard, when the pH was set at 5.0 or at 8.0 (Fig. 3). During a second set of experiments, the pH was changed from the standard set point value of 6.5 to other levels for a certain period, and then corrected back to 6.5. When the pH was changed during the first declining phase of the fermentation from 6.5 to 12, all specific rates were normal in the early period but after the pH was changed their values decreased as shown in Fig. 4. Changing the pH from 6.5 to 6.9 or 6.0 for three hours

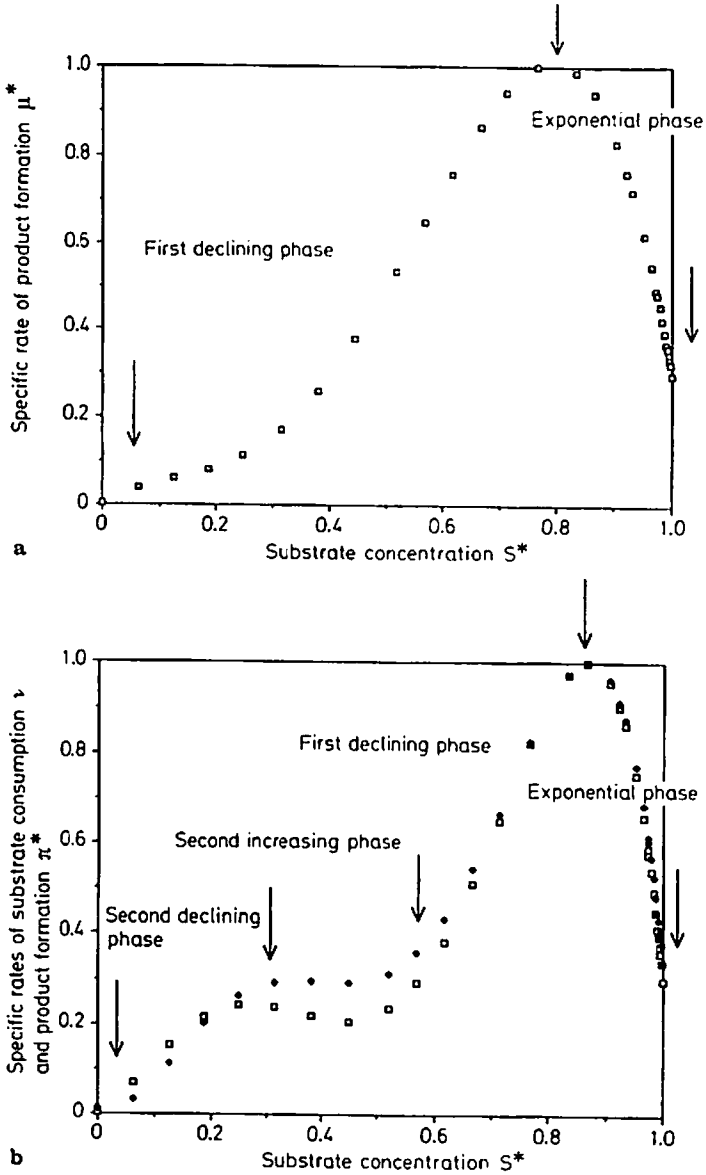

Fig. 2a, b. The different states of microbial activity a states of the specific rate of cell growth, b states of specific rates of substrate consumption and product formation

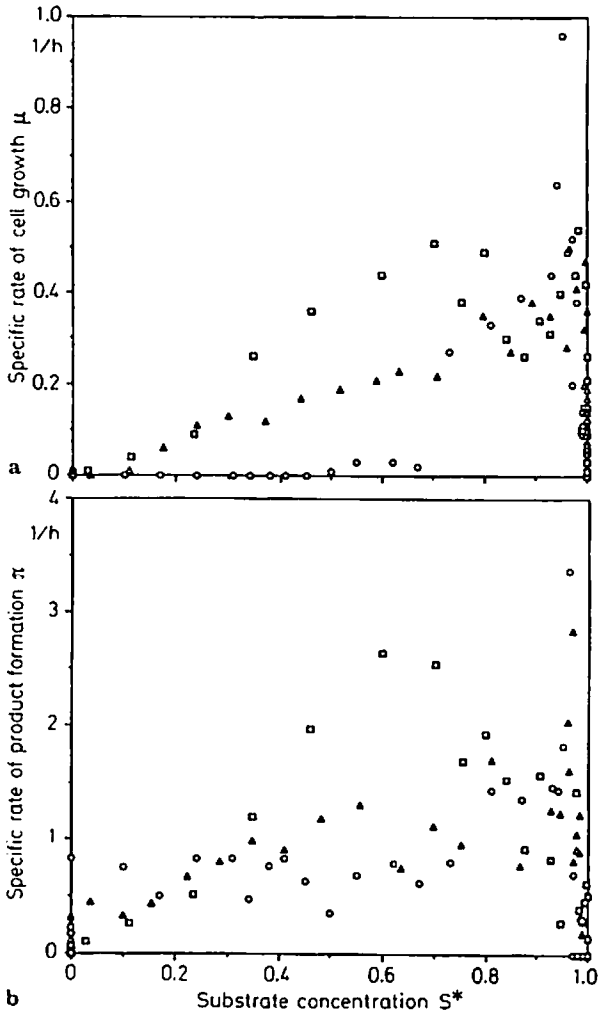

Fig. 3a, b. a The specific rate of cell growth and b the specific rate of product formation calculated from fermentations done with different pH setpoints (□) pH 5.0, (△) pH 6.5, (○) pH 8.0

during the exponential growth phase had only a small effect on the specific rates. The micro-organism could recover under these circumstances, after the pH was corrected back to normal (Fig. 5). Thus, the analysis of specific rates gave also information about the possible counter measures against a fault situation. The appropriate measure seemed to depend on the phase of the fermentation and the degree of the fault, and would vary from recovery from the fault to the termination of the fermentation. In comparison to the normal on-line monitoring of pH and off-line analysis of lactic acid, the specific rates also made possible to immediately recognize the effects of changes in process conditions on the product formation or cell growth. The microbial activities changed also from the standard levels for example when the age of the inoculum or the medium composition was altered.

5 Conclusions

The specific rates of growth, substrate consumption and product formation were calculated on-line to diagnose the microbial physiology during a fermentation process. The specific rate values seemed more sensitive to even slight changes in cultivation conditions such as pH than the corresponding real values of the cell mass, or substrate and product concentrations. The specific rates would offer more information about a fermentation process than what can be obtained by techniques based on the use of conventional software. The on-line monitoring of the specific rates provided a convenient way to diagnose the state of the fermentation processes. Further, the method is expected to detect contaminations.

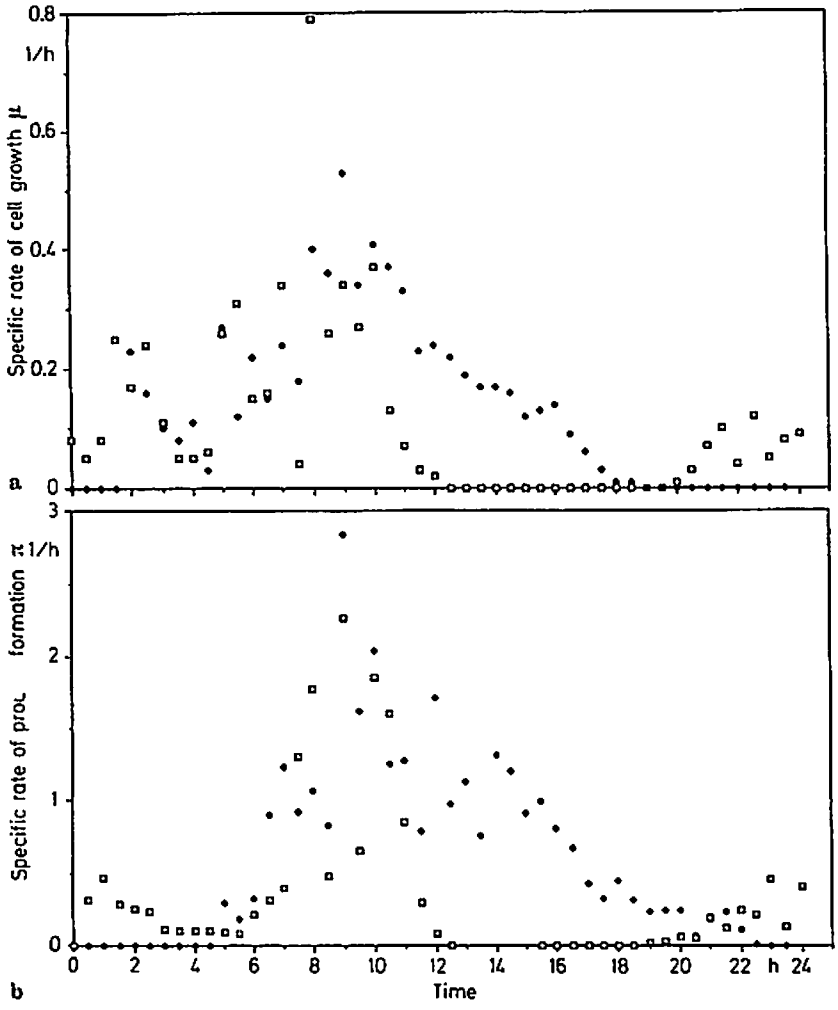

Fig. 4 a, b. The effect of the pH change from 6.5 to 12 at fermentation time 12 hours on a the specific rate of cell growth (•) pH normal 6.5, (▫) pH changed to 12. b the specific rate of product formation (•) pH normal 6.5, (▫) pH changed to 12

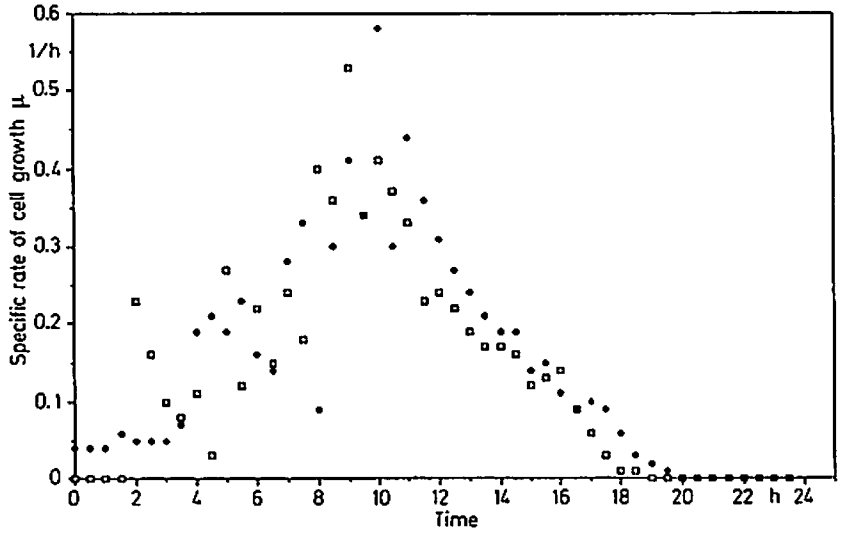

Fig. 5. The effect of the pH change from 6.5 to 6.9 for 3 hours at fermentation time 8 hours to the specific rate of cell growth (▫) pH normal 6.5, (•) pH changed to 6.9

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