A STABILIZATION OF ASPARTASE ACTIVITY OF IMMOLIZED *Escherichia coli* CELLS BY TEMPERATURE-RAISING OPERATION

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**Introduction**

In considering the economics and advantages of bio-catalysts, it is important to decide an optimal operation policy for biochemical reaction using immobilized enzymes and cells. Although optimal temperature policies have been widely reported concerning chemical reactors with catalyst decay, there are few papers about enzyme reactors.

Haas *et al.* reported the operational policy of optimal temperature and optimal isothermal temperature for the glucose isomerase reaction. Lim and Emigholz investigated optimal operation of a batch enzyme reactor using glucose isomerase. Pitcher described the operating strategy for immobilized lactase.

In the preceding paper the optimal operation policy of aspartase reaction by immobilized *Escherichia coli* cells was described, without regard to decay of aspartase activity. That is, in order to obtain higher conversion, it was necessary to decrease reaction temperature corresponding to the progress of the reaction near the end of the reaction.

The purpose of this paper is to discuss the effect of operation temperature on the operational stability of aspartase activity of immobilized *E. coli* cells, and to describe the operation policy while taking account of decay of the enzyme activity.

**1. Experimental**

**1.1 Apparatus used for operational stability of immobilized *E. coli* cells**

Figure 1 is a schematic diagram of a mini-column apparatus. Two and a half grams of immobilized *E. coli* (ATCC 11303) cells (19), prepared by the polyacrylamide method, were packed into 10 mini-columns (1-10, diameter: 2 cm, length: 15 cm) with outer jacket (18) evacuated to prevent heat conduction. Glass beads (20) were also packed above and below immobilized *E. coli* cells to obtain plug flow of a substrate solution. The substrate solution was fed by a multichannel peristaltic pump (22) at a flow rate of 50 ml/h from a stock tank (23) installed in a temperature-controlled box (11) and heated to prescribed temperatures with ribbon heaters (16) installed near the inlet of the mini-columns. The caloric value of the ribbon heaters was controlled by silicon-controlled rectifiers (14) fed from an automatic voltage regulator (13). Temperatures of the substrate solution in the mini-columns and that of the temperature-controlled box were recorded by a temperature recorder (15). The effluents from the mini-columns were collected via levelers (21) by an automatic multi-sampler (24).

The reaction temperature of the mini-columns was set to be 26, 30, 33, 35, 37, 40, 45, 50, 55 and 60°C, each of which was varied within ±0.2°C. One-molar ammonium fumarate solution containing 1 mol/m³ Mg²⁺ (pH 8.5) was used as a substrate solution.

**1.2 Determination of aspartase activity in column reactor**

Aspartase activity was calculated by Eqs. (1), (2) and (3) described in the preceding paper.

\[
V'_{am} = \frac{V}{t} \left\{ S_0 - S - \frac{K'_e K_{am}}{K'_e + 1} \ln \left( \frac{K'_e + 1}{S_0 K'_e} \right) \right\}
\]

(1)

\[
K'_e = -(3.603 S_0 - 0.284) T + 1200 S_0 - 78
\]

(2)

\[
K_{am} = 0.68 S_0^{1.04}
\]

(3)

where \(K_{am}\) is the apparent Michaelis constant, \(K'_e\) is the modified equilibrium constant, \(S\) is the concentration of fumaric acid, \(S_0\) is the input concentration of fumaric acid, \(T\) is the absolute temperature, \(t\) is the reaction time, \(V\) is the volume of substrate solution and \(V'_{am}\) is the apparent maximum reaction rate. Concentration of fumaric acid in column effluent was measured spectrophotometrically at
280 nm according to the method of Bock and Alberty.\textsuperscript{1)}

2. Results and Discussion

2.1 Operational stability of aspartase activity of immobilized \textit{E. coli} cells

Operational stability profiles at various reaction temperatures are given in Fig. 2. Aspartase activity of immobilized \textit{E. coli} cells decreased exponentially below 37°C during operational periods. However, the regression lines of exponential decay above 40°C showed broken behavior. In these cases, the aspartase activity decreased rapidly to 40–50% of its initial value. The reason for such initial decay is considered to arise from leakage of aspartase from gel matrices.

Rapid decay of aspartase activity was observed again when the activity became 10–20% of its initial value above 40°C, except in the data at 45°C. The reason for this phenomenon is not clear but can be explained by the decay due to the accumulation of toxic substances in the substrate solution, or the decrease of an unknown stabilizer within gel matrices.

Furthermore, the reason why such a phenomenon was not observed below 37°C can be considered as follows. Either the aspartase activity was not decreased to the critical value at which its rapid decay is initiated at the end of the experiment, or aspartase has a resistance to rapid decay below 37°C.

The decay constants below 37°C were calculated from the regression lines in Fig. 2 and are plotted against 1/\(T\) (Fig. 3). The decay constant is represented by Eq. (4):

\[
\text{Decay constant} = \exp\left(-9.39 \times 10^3/T + 25.29\right)
\]  (4)

Therefore, \(V_{am}\) is given as a function of \(T\) and operation time, \(\theta\), by Eq. (5):

\[
V_{am} = V_{am}^0 \exp\left(-\exp\left(-9.39 \times 10^3/T + 25.29\right)\theta\right)
\]  (5)

where \(V_{am}^0\) is the initial apparent maximum reaction rate.

2.2 Operation policy for industrial production of L-aspartic acid

Generally, one of the most important factors for cost estimation is the decay of enzyme activity of immobilized microorganisms and enzymes during operation. Temperature is a well-known factor affecting enzyme stability. When operating temperature is lower, enzyme activity is lower but more stable. Therefore, if the initial enzyme activity is sufficiently higher than the enzyme activity required to obtain the
desired conversion, the operating temperature can be set at a lower value till the enzyme activity falls to the required value to obtain desired conversion. Thus, we compared the efficiency of L-aspartic acid production between a temperature-raising operation and an isothermal operation on the basis of the rate equations described above. The results showed that the temperature-raising operation was obviously superior to the isothermal one. We are now industrially producing l-aspartic acid using immobilized *E. coli* cells with temperature-raising operation.

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**Nomenclature**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
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<tbody>
<tr>
<td>$K_{am}$</td>
<td>apparent Michaelis constant</td>
<td>[kmol/m³]</td>
</tr>
<tr>
<td>$K_2$</td>
<td>modified equilibrium constant</td>
<td>[-]</td>
</tr>
<tr>
<td>$S$</td>
<td>concentration of fumaric acid</td>
<td>[kmol/m³]</td>
</tr>
<tr>
<td>$S_o$</td>
<td>input concentration of fumaric acid</td>
<td>[kmol/m³]</td>
</tr>
<tr>
<td>$T$</td>
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<td>$t$</td>
<td>reaction time</td>
<td>[h]</td>
</tr>
<tr>
<td>$V$</td>
<td>volume of substrate solution</td>
<td>[l]</td>
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<tr>
<td>$V_{am}$</td>
<td>apparent maximum reaction rate</td>
<td>[mol/h·g-gel]</td>
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<tr>
<td>$\theta$</td>
<td>operation time</td>
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**Literature Cited**


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