INOSINE FERMENTATION—PRODUCTION OF INOSINE BY MUTANTS OF *BACILLUS SUBTILIS*

IV. SCALE-UP OF INOSINE FERMENTATION

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Using r_{ab} values, rates of oxygen transfer into sulfite solution, as an index for establishing aeration-agitation, the fermentative production of inosine was scaled up from a flask culture to a 50-kl tank culture. By applying the optimum condition for the production of inosine obtained with small scale fermentors, the yield of inosine obtained with a 50-kl fermentor was approximately the same as that from small-scale fermentors. With due consideration to cotton plugs as a barrier of oxygen transfer, the advantage of using r_{ab} values for aeration-agitation index and for scale-up of aerobic fermentation was discussed.

In the previous work, the production of inosine by mutants of *Bacillus subtilis* was studied in small flasks (1–3). An attempt was later made to enlarge the fermentation from a flask culture to a large-scale tank culture. This paper deals with some experimental data obtained during the preliminary course of industrial production of inosine. It has been demonstrated through this study that the optimum aeration-agitation condition is especially important in a large-scale fermentative production.

MATERIALS AND METHODS

Strain. A mutant of *Bacillus subtilis* K, C-30, requiring adenine, histidine and tyrosine for growth was used.

Medium. Cultivation medium consisted of glucose 6–7%, NH_4Cl 2%, dry yeast (Toyo Boseki Co., Ltd.) 1.4%, KH_2PO_4 0.1%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.04%, FeSO_4 2 ppm, MnSO_4 2 ppm and “Mieki” (aqueous solution of amino acid mixture prepared from HCl-hydrolysate of soybean meal, 20 g of nitrogen per liter) 8 ml/liter.

Fermentation. Cultivation was performed in geometrically equally designed fermentors with an operating volume of 5 liters, 10 liters, 1 kl and 50 kl. Sterile air was introduced into the fermentor from a sparger at the

bottom of the vessel, and agitation was made by a turbine impeller. Power was supplied from a constant-speed electric motor to the impeller. The shaft speed was adjusted by a belt-and-pulley system.

Analysis. The methods adopted for the analysis of glucose and inosine were described previously (1). Oxygen transfer was estimated by the sulfite oxidation method with gas analyzers (4-6) using the reaction mixture described by K. YAMADA (7).

Determination of oxygen transfer rate. For the determination of oxygen transfer rate from air into sulfite solution (5), it was necessary only to read the difference in oxygen tension between inlet and outlet gas streams of a fermentor filled with 0.8 N Na_2SO_3 and 0.001 M CuSO_4 . The following relationships were used in deriving the oxygen transfer rate:

$$r_{ab} = \left\{ Q_i \cdot \frac{273}{273 + t_i} \cdot P_i X_i - Q_o \cdot \frac{273}{273 + t_o} \cdot P_o X_o \right\} \cdot \frac{1}{22.4} \cdot \frac{1}{V} \cdot \frac{1}{10^3}$$

where r_{ab} is the oxygen transfer rate (mole/ml·min), Q is the air flow rate (l/min), P is the air pressure (atm), X is the oxygen concentration, and V is the liquid volume (liter). Subscripts i and o refer to inlet and outlet gas streams, respectively.

RESULTS

Aeration-agitation

The effect of aeration-agitation on the fermentative production of inosine in shaken flasks was discussed previously (3) from the aspect of oxygen absorption coefficient, Kd , in sulfite solution. It was commented also by one of the authors (Y. Hirose) that the use of r_{ab} values, rates of oxygen transfer (mole/ml·min), might be more practical for discussion, since better correlation was found in the glutamic acid fermentation between the product formation and the effectiveness of aeration when the term of r_{ab} was used instead of Kd (8).

Fig. 1 shows the relationship between inosine productivity expressed as percentage yield based on glucose added and the effectiveness of aeration-agitation in terms of sulfite r_{ab} . At the r_{ab} range from 5×10^{-7} to 12×10^{-7} , the three fermentors, 5-liter jar fermentor, 10-liter jar fermentor and 1-kl tank fermentor, yielded almost equal percentage of inosine. Below the r_{ab} value of $5-6 \times 10^{-7}$, that stands for Kd value of $2-3 \times 10^{-6}$ (mole/ml·min·atm), the inosine production increased with the increment of r_{ab} . This result is consistent with that obtained with shaken flasks charged with different volume of medium if the barrier of cotton plugs in oxygen transfer is taken into consideration (3).

pH and temperature

In the jar and tank cultures, pH could be adjusted to a constant level by

Fig. 1. Effect of sulfite r_{ab} on inosine productivity.

● 5-liter jar fermentor ○ 10-liter jar fermentor ▲ 1-kl tank fermentor

Fig. 2. Effect of pH on inosine productivity.

△ 10-liter jar fermentor ▲ 1-kl tank fermentor

automatic addition of gaseous ammonia. Fig. 2 shows the effect of pH on the production of inosine. The pH value optimum for the production was found at 6.0–6.2, indicating a good agreement with the results obtained with shaken flasks (3). Meanwhile, the pH range giving a high yield of inosine was narrower in the jar and tank cultures.

A high yield of inosine was obtained by incubation at 30° (Table 1).

Table 1. Effect of temperature on inosine productivity.

Temperature (°C)	Culture time (hr)	Inosine productivity (g/liter)	Yield based on glucose (%)
30	72	8.0	12.8
34	60	6.3	10.1
37	48	5.6	8.8

pH 6.0.

sulfite r_{ab} $6-7 \times 10^{-7}$ mole/ml·min.

Fig. 3. Time course of inosine production in a 50-kl tank fermentor.

Production of inosine in a 50-kl tank fermentor

A 50-kl tank fermentor was operated under the optimum condition described above; $r_{ab} = 11.8 \times 10^{-7}$ mole/ml·min (76 rpm, 1/4 v/v min), pH=6.0, temperature 30°. One example of the runs is shown in Fig. 3. The yield of inosine was about 14% and proximate to that obtained with smaller vessels.

DISCUSSION

The fermentative production of inosine was enlarged from a flask culture to a 50-kl tank culture. One important factor that made the scale-up successful may be the use of r_{ab} as an index for aeration-agitation. In shaking cultures, the critical value of Kd for the maximum production of inosine was

5.8×10^{-6} mole/ml·min·atm (3). This level of aeration was realized by shaking the flask with 40 ml of liquid volume, and oxygen transfer coefficient of cotton plugs employed was approximately 3×10^{-4} mole/min·atm (6). From these informations, the value of r_{ab} in sulfite solution was estimated to be about 6.9×10^{-7} mole/ml·min. Evidently, the critical value of r_{ab} was essentially the same in flask and jar fermentations, although that of Kd was not identical. The discrepancy of critical value of Kd might be due to the neglect of cotton plugs as a barrier of oxygen transfer as shown by one of the authors (Y. Hirose) in the glutamic acid fermentation (8). As a basis for scale-up of aerobic fermentations, sulfite oxidation method was employed in many cases such as yeast propagation (9–11) and bacterial cobalamin production (12). In some cases, however, this method was found to have poor correlation with the fermentation (13–15). The physical properties of sulfite solution differed appreciably from those of fermentation broths, and the value of the oxygen transfer rate measured by this method might not represent those applicable to fermentation (16). Most cases of unsuccessful scale-up based on this method were thought to be attributable to this physical difference. The results shown in this paper, however, may indicate another possibility that the barrier of oxygen transfer originating from cotton plugs was responsible for the discrepancy: consideration of cotton plugs from the point of oxygen transfer gave a good correlation of sulfite oxidation method with product formation both in flask cultures and in jar or tank cultures.

Nevertheless, the basic problem on the difference between sulfite solution and fermentation broths still remained to be solved; sulfite oxidation method could not be more than overall criterion of aeration-agitation conditions which should be discussed from the point of aeration and agitation independently. In order to avoid a possible failure of the scale-up which might result from these problems, the aeration-agitation condition of 50-kl tank cultures was, therefore, set at a fairly high level in the region expected to yield maximum productivity as shown in Fig. 1.

Although different in the methods of control, the pH value for maximum productivity was substantially the same in both shaken cultures (3) and in jar or tank operations. A more sensitive response to pH was observed in controlling with gaseous ammonia, but the precision of the control system did not cause any trouble in the performance. Moreover, dilution of culture broths, which was inevitable when controlled with liquid alkali and might cause serious disadvantages in foam control of the fermentation and in recovering the product, could be avoided by this method of control.

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