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Optimization of α-Galactosidase Production by Mold

(I) α-Galactosidase Production in Batch and Continuous Culture and A Kinetic Model for Enzyme Production

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Abstract

Monascus sp. newly isolated from soil was used for α-galactosidase production. α-Galactosidase is an intracellular enzyme induced by galactose, melibiose, raffinose, or stachyose. It was considered to be useful to employ glucose as a cheap carbon source for cell growth, followed by enzyme induction with galactose. Ammonium nitrate was used as the nitrogen source for enzyme production. The optimum conditions for cell growth and enzyme production were determined, as were the optimum conditions for enzyme reaction. When gulucose and galactose were supplied simultaneously, this mold utilized glucose before the initiation of galactose utilization for α-galactosidase production. Cell growth was diauxic.

Single-stage continuous culture of this mold was carried out and the empirical constants involved in the kinetic equation regarding cell growth and sugar consumption were assessed. When glucose was present at a concentration of more than 2.25×10^{-4} g/ml, glucose repressed galactose consumption and α-galactosidase production. Galactose competitively inhibited glucose consumption by this mold. The steady-state relationships in shift-down experiment were quite different from those in shift-up experiment. For the first time, "hysteresis" phenomena in continuous culture were observed. The authors proposed a kinetic model for the enzyme production, including intracellular reactions; the constants at the molecular level that could not be determined experimentally were estimated. Several kinetic models were compared with the experimental data from a single-stage continuous culture.

Introduction

It is well known in the beet sugar industry that raffinose inhibits the crystallization of sucrose. Suzuki *et al.*[1] have described a method by which raffinose was enzymatically decomposed by α-galactosidase-producing microorganisms. This method improved the crystallization efficiency and the yield of sucrose. The present authors isolated from soil a mold exhibiting an appreciable α-galactosidase activity, but with only a slight invertase activity. This paper deals with the effect of culture conditions on the α-galactosidase production. In addition a single-stage continuous culture was carried out with the object of measuring some of the empirical constants used in kinetic equations for cell growth and sugar consumption. Based on this work, the authors proposed a kinetic model for the enzyme production in this system.

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Materials and Methods

1. Strain The morphological characteristics of the isolated fungus grown on Koji extract agar were as follows: mycelium well developed, branched, and septate, forming conidia, cleistothecium, spores many; red pigment. This mold was identified, based on these results, as a *Monascus* sp., and was used in this work.
2. Media The growth medium was composed of 20 g of glucose, 5 g of NH_4NO_3, 5 g of KH_2PO_4, 1 g of $MgSO_4 \cdot 7H_2O$, and 0.1 g of yeast extract in 1000 ml of tap water, pH 4.5. The induction medium contained 5 g of galactose, 5 g of NH_4NO_3, 5 g of KH_2PO_4, and 1 g of $MgSO_4 \cdot 7H_2O$ in 1000 ml of tap water, pH 4.5.
3. Cultivations Batch cultures for the determination of optimum media composition were carried out in a 500 ml flask (liquid volume = 100 ml). The flask was shaken on a rotary shaker. Batch cultivations and continuous cultivations to examine cell growth and enzyme production were carried out in a 10 *l* jar fermentor (liquid volume = 4 *l*). The agitation speed was 500 rpm and the aeration rate was 1 vvm. Since the optimum temperatures for cell growth and enzyme production were 30°C and 35°C, respectively, these temperatures were adopted in this work. The optimum pH was the same, 4.5, for both cell growth and enzyme induction.
4. Crude enzyme solution Cells were harvested by filtration, washed with distilled water, and ground with quartz sand to a paste. The paste was suspended in an amount of 0.1 M acetate buffer (pH 4.5) equal to the original volume of the sample harvested and the suspension was used as crude enzyme solution. The enzyme was not inactivated under these conditions.
5. Enzyme assay α-Galactosidase was assayed by the modified Dey's method.[2] An appropriately diluted enzyme solution (0.2 ml) in acetate buffer (0.1 M, pH 4.5) was incubated at 50°C with 0.2 ml of 10 mM *p*-nitrophenyl-α-D-galactoside (PNPG) solution for 10 min. The reaction was stopped by adding 5.0 ml of a sodium carbonate solution (0.1 M). The solution was centrifuged (5000 rpm, 5 min) and the clear supernatant was assayed. The yellow color of *p*-nitrophenol thus developed was measured by determining the optical density at 405 mμ. A blank using inactivated enzyme (treated at 100°C, 3 min) was conducted in parallel and duly corrected for. The specified conditions gave a linear relationship between product formation and the amount of enzyme, or time of incubation, provided that the extinction of the final test solution did not exceed 0.85.

One unit of enzyme activity was defined as the amount of enzyme requried to hydrolyse 1 μmole of PNPG per min.

6. Determination of sugars Total sugars were determined by the phenol-sulfate method. Glucose and galactose were determined using the glucose oxidase, "glucostat", and galactose oxidase, "galactostat", methods. (Reagents were obtained from the Worthington Biochemical Corp., U.S.A.)
7. Determination of cell mass Cells were filtered and dried at 90°C for 24 hr, and weighed. A constant dry weight was achieved using this procedure.
8. Analysis of the kinetic model A digital computer was used for the analysis of the kinetic model. Constants at the molecular level that could not be determined experimentally were estimated by trial and error.

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Results and Discussion

1. The effect of pH and temperature on the enzyme reaction The effect of pH on the enzyme reaction was examined (Fig. 1–a). Acetate buffer (0.1 M) was used in this experiment. The optimum pH of α-galactosidase activity lies at pH 4.5. The effect of temperature on the enzyme reaction is shown in Fig. 1–b. The reaction at each temperature was carried out at pH 4.5 for 10 min. Apparent enzyme activity was the highest at 60°C. Since the enzyme might be inactivated at this temperature, the heat stability of the enzyme was examined (Fig. 1–c). Crude enzyme solution was maintained at each temperature for 30 min prior to the enzyme reaction at 30°C for 10 min. The enzyme was inactivated at 55°C or more in this condition. On the basis of this data, a temperature of 50°C was used for the measurement of enzyme activity.

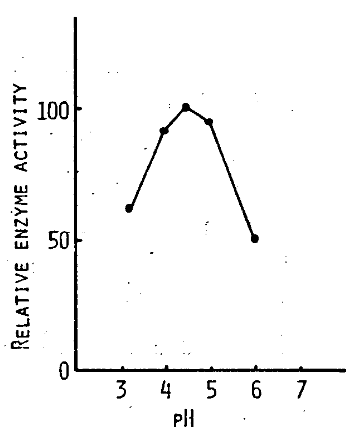

Fig. 1-a. Effect of pH on enzyme reaction. Acetate buffer (0.1 M) was used.

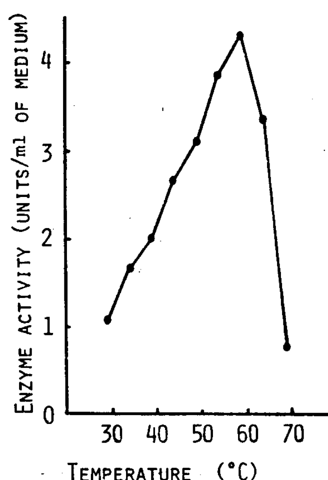

Fig. 1-b. Effect of temperature on the enzyme reaction. Enzyme reaction was carried out at each temperature at pH 4.5 for 10 min.

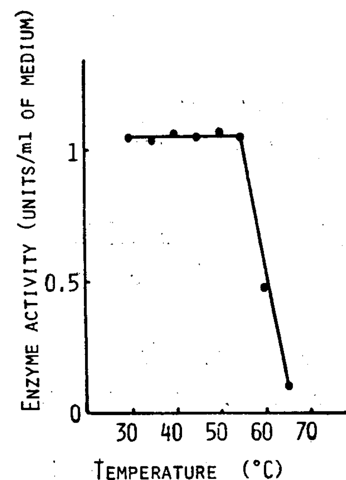

Fig. 1-c. Effect of heat treatment on enzyme inactivation. Crude enzyme solution was treated at each temperature for 30 min prior to the enzyme reaction at 30°C at pH 4.5 for 10 min.

2. Effect of carbon sources on α-galactosidase production To examine the effect of carbon sources on α-galactosidase production, various sugars were used instead of glucose in the growth medium. The relationship between carbon source and α-galactosidase production is shown in Fig. 2. Galactose, melibiose, raffinose, and stachyose induced α-galactosidase yielding high enzyme activity, while other sugars yielded very little α-galactosidase. Hognes *et al.*[3] reported that galactose and α-galactoside facilitated the α-galactosidase production of *Aerobacter aerogenes*. Suzuki *et al.*[4] reported that α-galactosidase of *Mortierella vinacea* and *Streptomyces* sp. were induced by galactose, lactose, melibiose, and raffinose. In this culture, most of the enzyme was present in the mycelium, while very little enzyme was present in the culture filtrate. Thus the α-galactosidase of this mold is an intracellular enzyme.

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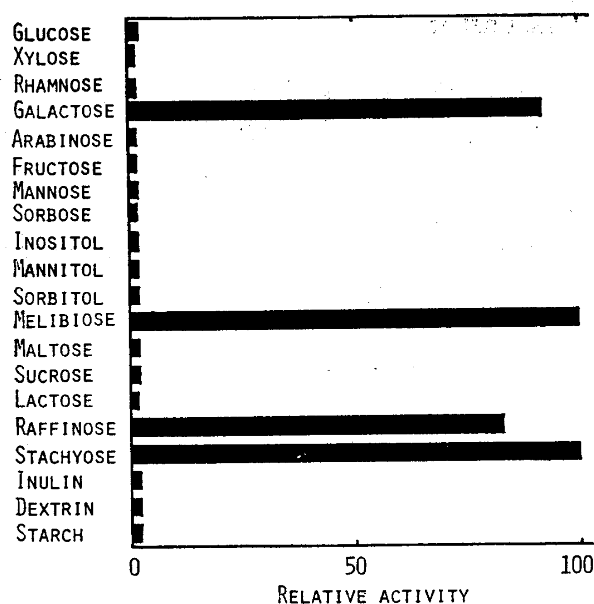

Fig. 2. α-Galactosidase production by *Monascus* sp. with various carbon sources.

Basal medium:

Sugar	2% (by wt)
NH_4NO_3	0.5%
KH_2PO_4	0.5%
$MgSO_4 \cdot 7H_2O$	0.1%
Temperature	30°C

3. Examination of the media and conditions for cell growth and enzyme production The effect of nitrogen sources on enzyme production is shown in Table 1. The concentration of the nitrogen source was 0.5% (by wt). Maximum relative enzyme activity was obtained with ammonium nitrate. The effect of ammonium nitrate concentration on enzyme production is shown in Table 2. The maximum specific enzyme activity was obtained with 0.3 and 0.5% of ammonium nitrate. The effect of galactose concentration on enzyme production is shown in Table 3. Cells grown in the growth medium at 30°C for 15 hr were filtered and

Table 1. Effect of the nitrogen source on enzyme production.

Nitrogen source	Relative enzyme activity
Peptone	100
$(NH_4)_2SO_4$	2
KNO_3	120
Urea	0
NH_4NO_3	135

Basal medium	
Galactose	1.0% (by wt)
KH_2PO_4	0.5%
$MgSO_4 \cdot 7H_2O$	0.1%
Nitrogen source added	0.5%
Tap water was used.	

Initial pH was 4.5, but pH was not controlled during batch culture.

Table 2. Effect of ammonium nitrate concentration on enzyme formation.

NH_4NO_3 (% by wt)	Cell mass (mg/ml)	Enzyme activity (units/ml of medium)	Specific enzyme activity (units/mg of cells)
0.1	10.40	1.50	0.144
0.3	11.78	5.76	0.489
0.5	11.35	5.53	0.487
1.0	11.20	5.10	0.455
2.0	11.10	4.88	0.439

Basal medium; galactose: 0.5% (by wt), KH_2PO_4: 0.5%, $MgSO_4 \cdot 7H_2O$: 0.1%. Temperature: 30°C. Tap water was used.
Initial pH was 4.5, but pH was not controlled during batch culture.
Cells grown in glucose medium were filtered, washed with distilled water, and transferred into the induction medium containing galactose.

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Table 3. Effect of galactose concentration on enzyme production.

Galactose concentration (% by wt)	Cell mass (mg/ml)	Enzyme activity (units/ml of medium)	Specific enzyme activity (units/mg of cells)
2.0	11.60	5.16	0.445
1.0	11.30	5.07	0.449
0.5	9.55	4.52	0.474
0.1	8.10	1.61	0.199
0.05	7.55	1.08	0.143
0.01	7.29	0.77	0.105

Basal medium for enzyme induction: NH_4NO_3, 0.5%; KH_2PO_4, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.1%.
Tap water was used. Temperature was 35°C.
Initial pH was 4.5, but pH was not controlled during enzyme production.
Cells grown in glucose medium were filtered, washed with distilled water, and transferred into the induction medium containing galactose.

washed with water and then transferred into the induction medium (35°C) containing various concentrations of galactose. The maximum specific enzyme activity was obtained at 0.5% galactose. The effect of metal ions on enzyme production was also examined. Zn^{++}, Ba^{++}, Ca^{++}, Fe^{++}, Co^{++}, Pb^{++}, and Mn^{++} affected enzyme production negatively.

The optimum temperatures for cell growth and enzyme production were 30°C and 35°C, respectively. The optimum pH for both cell growth and enzyme production was the same, 4.5, and in the range of pH 3.0 to 5.0, scarcely any differences in cell growth or enzyme production were observed.

4. Time courses of batch culture Glucose grown cells were filtered and used to inoculate a galactose medium (galactose was used instead of glucose in the growth medium). The time course of cell growth and α-galactosidase production on galactose is shown in Fig. 3. The pH was controlled with NaOH. The enzyme was produced in parallel with cell growth. Figure 4 shows the linear relationship

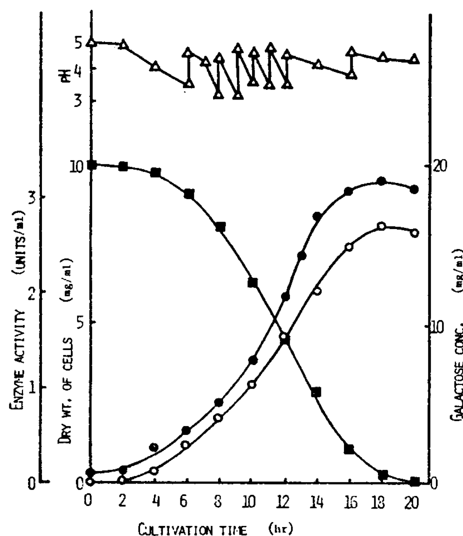

Fig. 3. Time course of cell growth and α-galactosidase production on galactose at 30°C.
■ galactose, ● cell mass, ○ α-galactosidase, △ pH.
The inoculum consisted of glucose grown cellls.

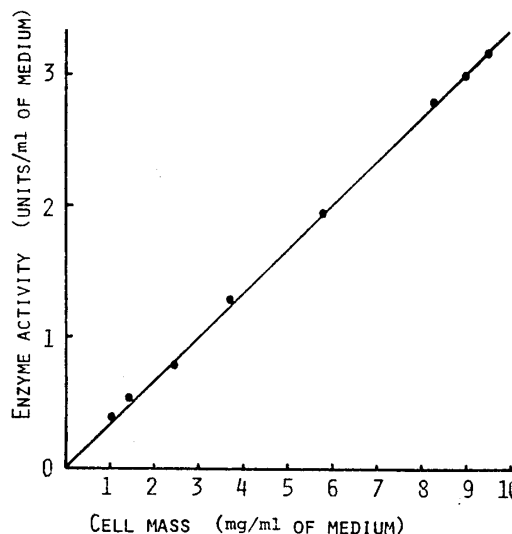

Fig. 4. Relationship between α-galactosidase in whole cells and the mass of the cells.
This data was obtained from batch culture with galactose.

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between α-galactosidase activity and the amount of cells during batch cultivation. As previously noted this α-galactosidase was an intracellular enzyme. Figure 5 shows the results of a batch culture in which a mixture of glucose and galactose was used as the carbon source instead of glucose alone. When glucose and galactose were thus supplied simultaneously, this mold utilized glucose before the initiation of galactose utilization and α-galactosidase production. When the glucose was nearly consumed, this mold began to utilize galactose and α-galactosidase was produced. The cell growth was typically diauxic.

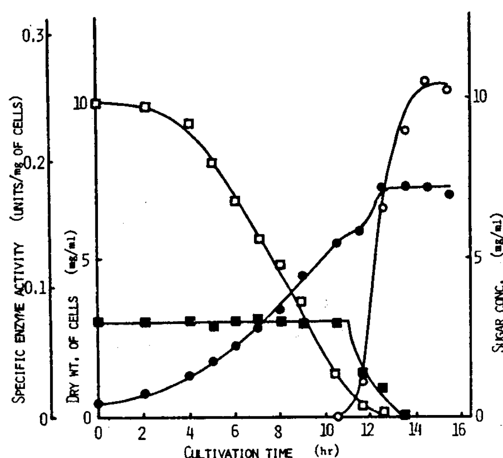

Fig. 5. Time course of cell growth and α-galactosidase production in the medium containing glucose and galactose.

The inoculum consisted of glucose grown cells. pH was controlled with NaOH.

Medium:

Galactose	0.3% (by wt)
Glucose	1%
NH_4NO_3	0.5%
KH_2PO_4	0.5%
$MgSO_4 \cdot 7H_2O$	0.1%
Yeast exract	0.01%

□ glucose ■ galactose ● cell mass ○ α-galactosidase

5. Enzyme induction and repression Enzyme induction by galactose and its repression by glucose were examined (Fig. 6). Cells grown in the glucose medium at 30°C for 15 hr were filtered and then transferred into the induction medium (35°C). This mold began to produce α-galactosidase 80 min later, and continued enzyme production for 2 hours. Glucose was added during the production of enzyme. The addition of 0.05% or more glucose apparently repressed the production of α-galactosidase.

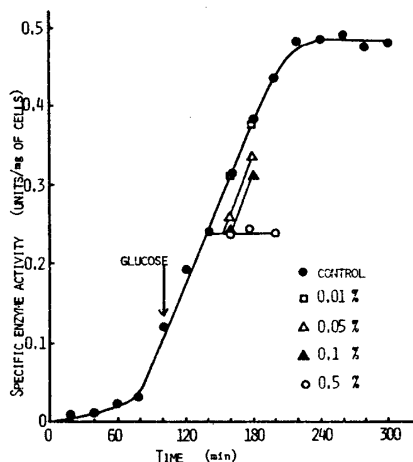

Fig. 6. Effect of glucose on α-galactosidase production.

Cells grown on glucose were filtered, washed with distilled water and placed into the induction medium. An arrow indicates glucose addition. Temperature: 35°C.

Induction medium:

Galactose	0.5% (by wt)
NH_4NO_3	0.5%
KH_2PO_4	0.5%
$MgSO_4 \cdot 7H_2O$	0.1%
pH	4.5

6. Single-stage continuous culture Knorre[5] examined the behavior of a β-galactosidase control system in continuous culture. Single-stage continuous cultures were carried out at 30°C or 35°C. Glucose or galactose was used as the carbon source. Continuous cultures of this mold conformed to Monod's equation under various growth conditions. The results are shown in Table 4. Glucose gives higher

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Table 4. Data of continuous cultivation at various conditions.

Carbon source	Temperature (°C)	μ_m (1/hr)	K_s (g/l)	Y
Glucose	30	0.215	0.154	0.530
Glucose	35	0.190	0.154	0.377
Galactose	30	0.208	0.258	0.516
Galactose	35	0.162	0.307	0.361

values of the maximum specific growth rate, μ_m, and cell yield constant, Y, and lower values of the saturation constant, K_s, than does galactose. When the same sugars were used, higher values of the maximum specific growth rate and cell yield and lower or unchanged values of the saturation constant were obtained at 30°C compared to 35°C. Figure 7 shows the results of a single-stage continuous culture in which 2% glucose and 0.5% galactose were used as carbon sources. When the dilution rate was changed from a low to a high value (shift-up experiment), a given set of steady-state values was obtained. One characteristic feature of this system is the discontinuity at a dilution rate equal to 0.142 1/hr. Another feature is that the "washout" state is attained at some dilution rate lower than the maximum specific growth rate of 0.215 1/hr. At dilution rates higher than 0.142 1/hr, only glucose was consumed; galactose was not and scarcely any α-galactosidase was produced. At a dilution rate lower than 0.142 1/hr, glucose and galactose were consumed together and α-galactosidase was produced. From these data, it would seem that when glucose is present at more than the critical concentration, glucose represses galactose consumption and enzyme production. Lineweaver-Burk plots of cell growth are shown in Fig. 8. It was found that galactose competitively inhibits glucose consumption by this mold. The inhibitor constant of galactose in the system, K_i, was 0.139 g of galactose/l. When the dilution rate was changed from a high to a low value (shift-down experiment), a different set of steady-state values

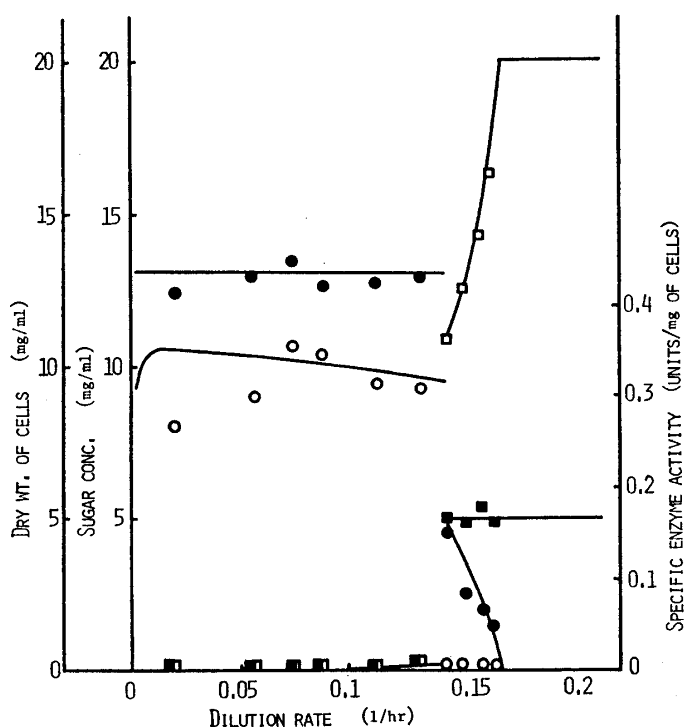

Fig. 7. Steady-state relationships in a single-stage continuous culture at 30°C (shift-up experiment).
□ glucose ■ galactose
● cell mass ○ α-galactosidase
——: calculation
The 2% of glucose and 0.5% of galactose were used as carbon sources.

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were obtained (Fig. 9). In this case, a discontinuity also appeared on several of the curves. At a higher dilution rate than 0.008 1/hr, glucose consumption was competitively inhibited by galactose, galactose was not consumed, and scarcely any α-galactosidase was produced. At dilution rates lower than 0.008 1/hr, glucose and galactose were consumed together and α-galactosidase was produced. The steady-state values obtained in the shift-down experiment were completely different from those obtained in the shift-up experiment. Figure 10 shows the relationships between sugar concentration and dilution rate. Different sugar concentrations in shift-down and shift-up experiments were obtained at identical dilution rates. This is the

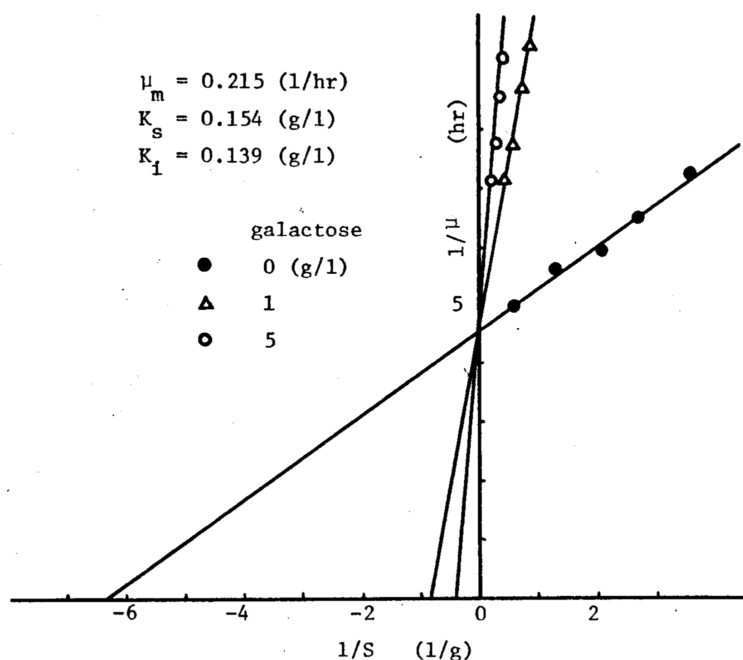

Fig. 8. Competitive inhibition of cell growth by galactose.

Experimental data were obtained from single-stage continuous cultures in which 2% of glucose and various amounts of galactose were used as carbon sources as indicated in the figure. pH: 4.5, temperature: 30°C.

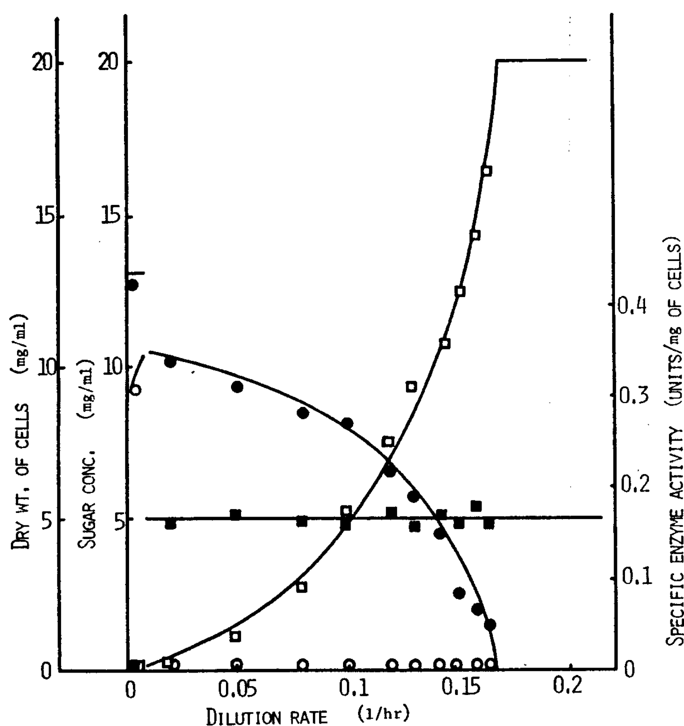

Fig. 9. Steady-state relationships in a single-stage continuous culture at 30°C (shift-down experiment).

The 2% of glucose and 0.5% of galactose were used as carbon sources.

□ glucose ■ galactose
● cell mass ○ α-galactosidase
——: calculation

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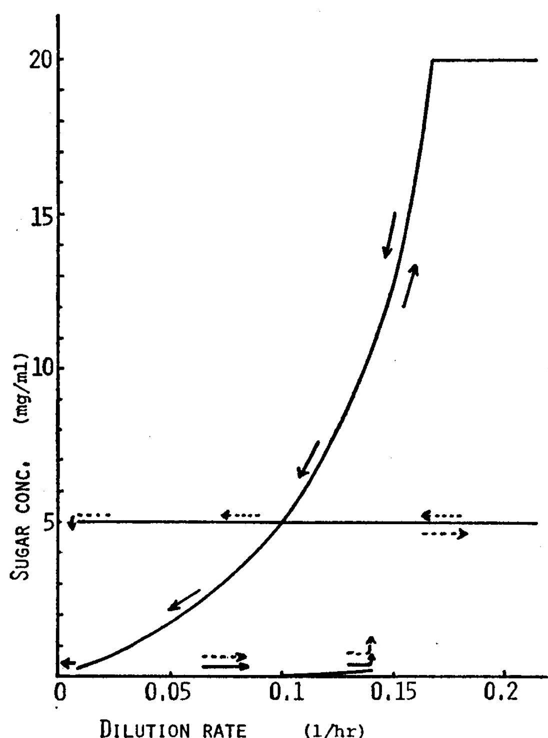

Fig. 10. Steady-state relationships between sugar concentrations and dilution rate.

This is the "hysteresis phenomena in continuous culture".

⟵: glucose ⇠: galactose

"hysteresis" phenomena in continuous culture. To change from a shift-down steady-state value to a shift-up steady-state value, the dilution rate of the continuous culture must be decreased to 0.008 1/hr or less so that the glucose concentration in the medium is below the critical value of 2.25×10^{-4} g/ml. The dilution rate may then be increased to its initial value.

7. Kinetic model of cell growth The following kinetic model of cell growth is proposed. The cell growth and sugar consumption rates are essentially represented by Monod's equation. Thus, the specific growth rate of cells, μ, can be defined as

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

With respect to glucose consumption, the term of competitive inhibition by galactose was added. The rate of glucose consumption is expressed as follows.

$$\frac{dS_A}{dt}=-\frac{X}{Y_A}\cdot\frac{\mu_{mA}S_A}{K_{SA}+S_A+\frac{K_{SA}}{K_i}S_B}=-\frac{\mu_A}{Y_A}X \tag{2}$$

Where X = cell concentration
S = sugar concentration
μ_m = maximum value of the specific growth rate
K_S = saturation constant
K_i = equilibrium constant in the system between cells and galactose
Y = yield constant
subscripts: A = glucose; B = galactose

The rate of galactose consumption is expressed as follows;

$$\frac{dS_B}{dt}=-\frac{X}{Y_B}\cdot\frac{\mu_{mB}S_B}{K_{SB}+S_B}=-\frac{\mu_B}{Y_B}X \tag{3}$$

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The over-all rate of cell growth, originating both from glucose and galactose, is shown below.

$$\frac{dX}{dt}=X\left\{\frac{\mu_{mA}S_A}{K_{SA}+S_A+\frac{K_{SA}}{K_i}S_B}+\frac{\mu_{mB}S_B}{K_{SB}+S_B}\right\}$$
$$=(\mu_A+\mu_B)X=\mu X \tag{4}$$

S_{AC} is the critical concentration of glucose beyond which galactose utilization is inhibited. When $S_A \geqq S_{AC}$, galactose remained unmetabolized. Then, $\mu_{mB}=0$.

8. Kinetic model of α-galactosidase production The kinetic model for α-galactosidase synthesis is based on the generally accepted characteristics of the operon model. It was assumed that the incorporation rate of galactose into the cell is expressed in a Monod type equation and galactose is consumed in the cell by a monomolecular reaction. The specific rate of change of galactose concentration in the cell is as follows:

$$\frac{1}{X}\cdot\frac{d(S_{Bi}X)}{dt}=\frac{1}{X}\left\{S_{Bi}\frac{dX}{dt}+X\frac{dS_{Bi}}{dt}\right\}$$
$$=U\left\{\frac{G_B S_B}{K_{mB}+S_B}-S_{Bi}\right\}-k_1 S_{Bi}$$

$$\frac{dS_{Bi}}{dt}=U\left\{\frac{G_B S_B}{K_{mB}+S_B}-S_{Bi}\right\}-k_1 S_{Bi}-\mu S_{Bi} \tag{5}$$

where U=constant G=constant K_m=constant
subscript i=intracellular

When $S_A \geqq S_{AC}$, galactose cannot be incorporated into the cell. Then, $U=0$.

It was assumed that the rate of repressor formation is constant in the cell. Since the repressor, R, was presumably decomposed by a monomolecular reaction, and was reversibly combined with inducer, attaining eventually an equilibrium with galactose, the specific repressor formation rate is:

$$\frac{dR}{dt}=k_2-k_3R-k_4RS_{Bi}+k_5\overline{RS_{Bi}}-\mu R \tag{6}$$

where $\overline{RS_{Bi}}$=amount of the complex of repressor and inducer (galactose).

The mRNA, M, is decomposed by a monomolecular reaction. Assuming that the rate of formation for mRNA is proportional to the difference between the repressor concentration and the critical value, R_c, the following equation is similarly obtained.

$$\frac{dM}{dt}=k_6(R_c-R)-k_7M-\mu M \tag{7}$$

When $R \geqq R_c$, R is taken as equal to R_c.

The enzyme formation rate is proportional to the concentration of mRNA. Since the enzyme was stable under the experimental conditions, the enzyme decomposition was considered negligible. The enzyme formation rate is thus;

$$\frac{dE}{dt}=k_8M-\mu E \tag{8}$$

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The rate of change of the concentration of the galactose-repressor complex is given by:

$$\frac{d\overline{RS_{Bi}}}{dt}=k_4 RS_B - k_5\overline{RS_{Bi}} - \mu\overline{RS_{Bi}} \quad (9)$$

where k_j $(j=1, 2, \cdots, 7, 8)$ = reaction rate constants.

9. Comparison of this model with experimental data This model was analysed with a digital computer by using the various values given in Table 5. These values were established by trial and error and are relative values. Novick *et al.*[6] reported that the repressor was "growth-unstable" with a mean life of 1/10 to 1/5 of a generation. When the specific growth rate is 0.215 1/hr, the generation time is about 200 min. The value for k_3 was determined on the supposition that the half-life of the repressor is 40 min and k_7 was determined on the assumption that the half-life of mRNA is 5 min. Calculations for the single-stage continuous culture, when glucose and galactose concentration in the incoming medium are 2% and 0.5%, respectively, were shown in Figs. 7, 9, and 10. It was calculated that the critical glucose concentration was 2.25×10^{-4} g/ml. This value is not in disagreement with the data in Fig. 6. The calculated values agreed fairly well with the continuous calture data.

Table 5. Various values used for calculation of this model.

k_1 =40	(1/hr)	*μ_{mGA} =0.215	(1/hr)
k_2 =1	(mg/mg of cells·hr)	*μ_{mGB} =0.208	(1/hr)
k_3 =1	(1/hr)	*μ_{mPA} =0.190	(1/hr)
k_4 =0.1	(mg of cells/mg·hr)	*μ_{mPB} =0.162	(1/hr)
k_5 =1×10^{-4}	(1/hr)	*K_{sGA}=1.54×10^{-4}	(g/ml)
k_6 =1	(1/hr)	*K_{sGB}=2.58×10^{-4}	(g/ml)
k_7 =8	(1/hr)	*K_{sPA}=1.45×10^{-4}	(g/ml)
k_8 =3.2787	(units/mg of M·hr)	*K_{sPB}=3.07×10^{-4}	(g/ml)
k_{8p} =5.0442	(units/mg of M·hr)	*K_i =1.39×10^{-4}	(g/ml)
S_{AC} =2.25×10^{-4}	(g/ml)		
U =100	(1/hr)	*Y_{GA} =0.530	
G_B =1	(mg/mg of cells)	*Y_{GB} =0.516	
K_m =1×10^{-8}	(mg/mg of cells)	*Y_{PA} =0.377	
R_c =0.934	(mg/mg of cells)	*Y_{PB} =0.361	

Subscripts G=condition of the cell growth.
P=condition of the enzyme production.
*: Experimental values.

Since galactose, melibiose, raffinose, and stachyose are expensive, it was considered to be useful to employ glucose as a cheap carbon source for cell growth, followed by the enzyme induction with galactose. To increase the productivity of enzyme, multi-stage continuous cultures are practical: cell growth in the first vessel, enzyme induction and production in the second vessel. A more exact analysis of the transient state of batch and continuous culture will be discussed in a later paper.

10. Comparison of this model with other models This kinetic model of enzyme production was compared with some other models. These models are as follows:

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(a) The case in which the amount of galactose in the cell is proportional to that in the medium at a steady-state condition.

$$S_{Bi}=G_B S_B \qquad (10$$

A typical pattern of specific enzyme activity is shown in Fig. 11. At low dilution rates, the specific enzyme activity is lower in the model than in the experimental data. This model is not suitable for the system.

(b) The case in which the rate of the active repressor formation is in proportion to the amount of glucose within the cell.

$$\frac{dR}{dt}=k_2 S_{Ai}-k_3 R-k_4 RS_{Bi}+k_5 \overline{RS_{Bi}}-\mu R \qquad (6)'$$

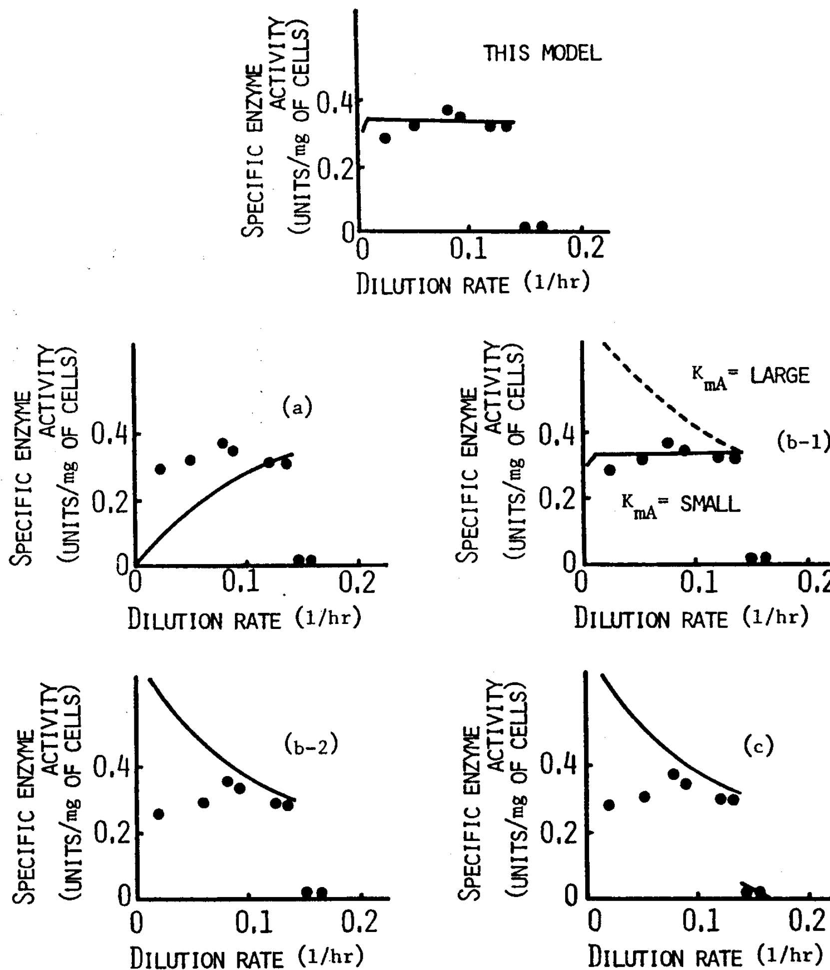

Fig. 11. Comparison of this model with others.

These models were analysed with a digital computer.

(a) Galactose amount in the cell is proportional to that in the medium.

(b) The rate of the active repressor formation is in proportion to the amount of glucose within the cell.

(b-1) Glucose amount in the cell was assumed to follow a Monod type equation with respect to glucose concentration in the medium.

(b-2) Glucose amount in the cell is proportional to that in the medium.

(c) The rate of mRNA formation is inversely proportional to the repressor concentration.

——: calculation data ●: experimental data

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(b-1) The case in which the amount of glucose in the cell was assumed to follow a Monod type equation for the glucose concentration in the medium (Fig. 11).

$$S_{Ai}=\frac{G_A S_A}{K_{mA}+S_A} \tag{11}$$

When K_{mA} is large, this model does not agree with the experimental data for this enzyme formation system. When K_{mA} is very small, the calculated data agrees well with the experimental data of continuous culture, but this model cannot adequately express the results of a batch culture because this model predicts infinite enzyme production in the stationary phase.

(b-2) The case in which the amount of glucose in the cell is proportional to that in the medium (Fig. 11).

$$S_{Ai}=G_A S_A \tag{11)'$$

This model does not fit the experimental data well.

(c) The case of Novick's model,[6] suggesting that the rate of formation for mRNA is inversely proportional to the repressor amount (Fig. 11).

$$\frac{dM}{dt}=k_6\frac{K}{K+R}-k_7 M-\mu M \tag{7)'$$

where K=constant. This model does not agree with the experimental data.

Nomenclature

E =specific enzyme activity (units/mg of cells)
G =constant (mg/mg of cells)
K =constant (mg/mg of cells)
K_i =inhibitor constant (g/ml)
K_m =constant (mg/mg of cells)
K_S =saturation constant (g/ml)
k_j $(j=1, 2, \cdots, 8)$=reaction rate constants
M =amount of mRNA (mg/mg of cells)
R =amount of repressor (mg/mg of cells)
$\overline{RS_{Bi}}$=amount of complex of repressor and galactose (mg/mg of cells)
S_i =intracellular amount of sugar (mg/mg of cells)
S =sugar concentration (g/ml)
U =constant (1/hr)
X =cell concentration (g of cells/ml of medium)
Y =yield constant

Subscripts: A=glucose
B=galactose
c =critical value
i =intracellular
m=maximum value

Greek letters: μ =specific growth rate (1/hr)

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