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https://doi.org/10.5109/23959

出版情報：九州大学大学院農学研究院紀要. 35 (3/4), pp.131-150, 1991-02. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 35 (3•4), 131–150 (1991)

Study on Batch Culture Growth Model for *Lactococcus lactis IO-1*

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(Received October 24, 1990)

L-lactate fermentation employing *Lactococcus lactis* IO-1 demonstrated a typical end product inhibition. By numerical analysis of fermentation results of the batch culture of this microorganism, the specific rates for cell growth, substrate consumption and product formation were clearly expressed by the end product inhibition formulae. All constants for those formulae were determined by the fermentation results. A mathematical model for batch culture growth of this microorganism in which the newly defined term α, a ratio of sterile cell formed to total cell population measured by 0. D., was used was constructed. By using this model, computer simulation was carried out and the batch culture growth gave satisfactory approximation to the observed results on the viable cell basis. However, the model gave a deviation from the observed results at the end phase of the fermentation. Further experiment showed that LDH activity in broth, the key enzyme of this fermentation, decreased with a constant decreasing rate which is smaller than α. Simulation program using enzyme decreasing rate gave perfect results that agreed with the observed results for the whole phase of the fermentation.

INTRODUCTION

Almost all fermentation kinetics are studied based on the continuous culture and no comprehensive rule has been established to the batch culture (Stunbury and Whitaker, 1984, Pirt, 1985), although batch culture is still the most common practice for industrial application. It is an easy matter to identify the mathematical parameters for the steady state of a continuous culture while the kinetic parameters of a batch culture are hardly set because a batch culture varies in fermentation dynamics time to time. It is therefore difficult to build a mathematical model to express batch culture fermentation dynamics and the kinetic study for batch culture remains unformed. There are many approaches to solve this subject including fussy reasoning (Rauman-Aalto and Linko, 1987). Those works are mainly used lactate fermentations (Rogers et al., 1978 and Jørgensen and Nikolajsen, 1987) and ethanol fermentations (Rogers, 1979 and Kosaric et al., 1984) as models.

Lactococcus lactis IO-1 is an L-lactate producer isolated in our laboratory which is capable of growing at relatively high temperature as 37°C (Ishizaki et al., 1990). The fermentation dynamics of this microorganism were substantially influenced by lactate concentration so that this fermentation showed a typical end product inhibition of uncompetitive inhibition formulae (Ishizaki and Ohta 1989). Hence, any biochemical reaction occurred during the fermentation is a result of the integration of multi-enzyme systems and the batch fermentation kinetics should be expressed by any kind

of enzyme kinetics formula. The Monod equation is too simple to express complicated course of fermentation. Yamane (1978) and Pirt (1985) predicted that kinetics of some trophophase fermentations may be expressed in the noncompetitive inhibition of the end product, however, no one has proved this in a culture system. Kosaric et al. (1984) introduced a mathematical model for ethanol fermentation using *Z. anaerobia* and they introduced a model based on the theory of product inhibition. However, their approach was based on statistics but not based on biochemistry.

We numerically analyzed the data obtained in the fermentation and discovered the comprehensive rules to express batch culture kinetics based on the enzyme kinetics of uncompetitive inhibition by the end product. The results showed that L-lactate fermentation of *L. lactis* 10-l is substantially controlled by the L-lactate concentration in broth. Thus the kinetics of batch culture in this fermentation can be expressed by the uncompetitive inhibition enzyme kinetics formulae (Ishizaki and Ohta, 1989). Kinetic parameters of the formulae were and used to build up a series of mathematical models to express the dynamic state of batch fermentation. Through this work, it was proved that the growth of *L. Zactis* IO-1 can be precisely predicted under given initial conditions using the term α which was defined for expressing a ratio of sterile cells formed to the total cell population (Ishizaki et al., 1989). However, at the end phase of the fermentation of a high initial sugar concentration, the simulation for substrate consumption and product formation formed a deviation from the observed results and a larger deviation developed with fermentation time. The deviation may have developed from the rate of enzyme inactivation, which is suspected to be smaller than the rate of sterile cell formation α. To prove this assumption, we measured the enzyme activity change in the culturing broth of this microorganism. Through this work, we completed the computer simulation program for L-lactate batch fermentation using *L. Zactis* IO-1 (Ishizaki and Kobayashi, 1990).

MATERIALS AND METHODS

Microorganism

Microorganism used was a *Lactococcus lactis* IO-1 (JCM 7638), which is a new strain of L-lactic acid producing coccus isolated in our laboratory (Ishizaki et al. 1990).

Medium

Medium for fermentation consisted of 5.0 g of yeast extract, 5.0 g of polypeptone, 5.0 g of sodium chloride and 1.0 l of distilled water. Three different glucose concentrations between 10 gl^{-1} and 80 gl^{-1} were used. The same medium with $10\,gl^{-1}$ glucose was used for inoculum preparation.

Culture method

Cultivation was carried out by a 1000 ml mini-jar-fermentor with 400 ml of medium under the agitation strength of 500 rpm without gas flow. The temperature was regulated at 37°C by the circulation of temperature controlled water. The pH was maintained at 6.0 with N-NaOH feeding.

Analysis

Chemical analysis was carried out as follows. Cell density was measured by optical absorbance at 562nm by using a spectrophotometer (Uvidec-320 Jas Co. Japan). The cells were collected by centrifugation, washed with distilled water and centrifuged again then dried at 105°C to constant weight to determine dry cell weight. A standard curve was drawn by plotting the optical density *versus* dry cell weight. Cell growth was indicated by calculating the dry cell weight according to the standard curve. Residual glucose in the broth and lactate in broth were determined by a glucose analyzer model 27 and a lactate analyser model 23L (YSI, Co., Ltd. USA) respectively.

Viable cell count

Viable cell count (CFU) was carried out by the method of Roy et *al. (1986)* using TGC agar (Difco) plate at the culture temperature of 37°C.

LDH assay

Lactate dehydrogenase (LDH) activity in culture system was assayed by a " Lactate dehydrogenase CII-Test Wako" kit purchased from Wako Pure Chemical Industries, Ltd. Osaka Japan (Babson and Phillips, 1965 and Kanai and Kanai, 1983). Since the LDH activity of broth without cell separation was approximately the LDH activity of broth whose cells were digested by lyzozyme and sonication, it was proved that the LDH activity of broth with whole cells represent the LDH activity of the culture system.

Data processing and data analysis

For numerical analysis, the analytical data were proportionally compensated for by using the amount of volume change of the culture liquid caused by the addition of neutralizing agent and sampling during fermentation. The concentration with the words "in broth" indicates the analytical results without compensation while the concentration without the words "in broth" indicates the data compensated so that the glucose concentration in broth and the lactate concentration in broth indicate analytical results without compensation, while the dry cell weight concentration, glucose concentration and lactate concentration without "in broth" indicate the results compensated for by the procedure stated. The specific growth rate, the specific glucose consumption rate, the specific lactate production rate and the yield factor for lactate from glucose were calculated using the following formulae,

$$\mu = \frac{\mathrm{d}X}{X\mathrm{d}t} \tag{1}$$

$$\mu_{\mathrm{S}} = \frac{\mathrm{d}S}{X\mathrm{d}t} \tag{2}$$

$$\mu_{\mathrm{L}} = \frac{\mathrm{d}L}{X\mathrm{dt}} \tag{3}$$

$$d\,Y_{L/S} = \frac{dL}{dS} \tag{4}$$

where μ is the specific growth rate, μ_S is the specific glucose consumption rate, μ_L is the specific lactate production rate, X is the dry cell weight concentration, S is the glucose concentration, L is the lactate concentration, $d\,Y_{L/S}$ is the differential yield factor for lactate from glucose and t is time. However for the calculation of fermentation kinetics in order to study the inhibitory effect of L-lactate as the end product, the concentrations of glucose in the broth and lactate in the broth were used for the

Table 1. Analytical results and calculation values for the fermentation of 10 $g l^{-1}$ initial glucose

Time h	X $g l^{-1}$	μ h^{-1}	S_b $g l^{-1}$	S $g l^{-1}$	dS/dt $g l^{-1} h^{-1}$	μ_S h^{-1}	L_b $g l^{-1}$	L $g l^{-1}$	μ_L h^{-1}	$d\,Y_{L/S}$
0	0.009		10.36	10.36			0	0		
2		0.200			0.461	30.70			2.12	0.069
4	0.021		9.01	8.52			0.13	0.13		
4.5		1.023			0.569	13.23			9.42	0.712
5	0.065		8.62	7.95			0.58	0.53		
5.5		1.244			0.663	3.86			3.51	0.911
6	0.279		8.02	7.29			1.25	1.14		
6.5		0.844			2.726	5.65			5.25	0.930
7	0.686		4.99	4.56			4.02	3.67		
8		0.264			2.280	2.45			2.31	0.944
9	1.179		0	0			8.59	7.98		

Table 2. Analytical results and calculation values for the fermentation of 50 $g l^{-1}$ initial glucose

Time h	X $g l^{-1}$	μ h^{-1}	S_b $g l^{-1}$	S $g l^{-1}$	dS/dt $g l^{-1} h^{-1}$	μ_S h^{-1}	L_b $g l^{-1}$	L $g l^{-1}$	μ_L h^{-1}	$d\,Y_{L/S}$
0	0.007		47.50	47.50			0	0		
3.5		0.152			0.844	58.92			1.44	0.024
7	0.023		43.84	41.31			0.16	0.15		
7.5		1.115			2.046	39.35			7.50	0.191
8	0.081		42.70	39.26			0.59	0.54		
8.5		1.232			1.397	6.62			5.13	0.775
9	0.341		42.12	37.87			1.81	1.62		
9.5		0.790			3.846	6.83			4.68	0.686
10	0.786		37.82	34.02			4.74	4.26		
10.5		0.542			4.806	4.46			4.13	0.927
11	1.371		31.42	29.21			9.38	8.72		
11.5		0.093			4.880	3.39			3.01	0.887
12	1.505		25.64	24.33			13.75	13.05		
17.5		0.019			1.839	1.10			1.02	0.933
23	1.855		3.60	4.10			28.02	31.94		
25		−0.008			1.025	0.56			0.57	1.013
27	1.789		0	0			30.94	36.09		

substrate and inhibitor concentrations. Calculation in terms of the value to influence material balance such as specific rate and differential yield factor was carried out using the concentration compensated. However for kinetics study under the influences of inhibitory effect of L-lactate, the concentration in broth was used for the substrate and the inhibitor concentrations. All calculations were carried out by the computer using the program self-prepared.

RESULTS AND DISCUSSION

Fermentation results and data analysis

Fermentation results

Fermentation results with data processing and calculations for three different initial sugar concentrations are presented in Tables 1, 2 and 3, respectively. Lower

Table 3. Analytical results and calculation values for the fermentation of 80 g l^{-1} initial glucose

Time h	X g l^{-1}	μ h^{-1}	S_b g l^{-1}	S g l^{-1}	dS/dt g $l^{-1}h^{-1}$	μ_s h^{-1}	L_b g l^{-1}	L g l^{-1}	μ_L h^{-1}	$dY_{L/s}$
0	0.006		83.75	83.75			0	0		
2.5		0.200			1.002	83.50			10.85	0.130
5	0.018		82.10	78.74			0.68	0.65		
55		1.053			0.820	21.58			8.55	0.396
6'	0.058		82.60	77.92			1.03	0.98		
6.5		1.101			1.910	14.81			5.55	0.375
7	0.200		81.55	76.01			1.82	1.69		
7.5		0.710			3.320	10.71			6.44	0.601
8	0.420		77.75	72.69			3.94	3.69		
8.5		0.579			3.800	6.43			4.95	0.770
9	0.762		72.65	68.89			6.98	6.61		
9.5		0.317			4.540	5.01			4.43	0.884
10	1.049		66.10	64.35			10.92	10.63		
10.5		0.179			3.890	3.38			3.29	0.976
11	1.255		60.10	60.46			14.34	14.42		
11.5		− 0.021			1.430	1.15			2.30	2.001
12	1.229		57.95	59.03			16.97	17.28		
18		0.029			1.624	1.09			0.99	0.905
24	1.745		33.35	39.54			29.45	34.92		
26		− 0.006			1.276	0.74			0.53	0.722
28	1.704		28.30	34.44			31.72	38.60		
29		0.014			1.412	0.81			0.60	0.731
30	1.751		25.70	31.61			33.06	40.66		
31		− 0.010			0.684	0.39			0.56	1.427
32	1.715		24.35	30.25			34.31	42.61		
39.5		0.005			0.821	0.46			0.44	0.960
47	1.844		13.15	17.94			39.91	54.43		
51		− 0.001			0.565	0.31			0.31	1.008
55	1.835		9.55	13.42			41.99	58.99		
63		− 0.009			0.511	0.30			0.35	1.180
71	1.596		3.49	5.24			45.69	68.64		
78		− 0.003			0.315	0.20			0.20	0.983
85	1.537		0.54	0.83			47.66	72.98		

fermentation rate at higher initial sugar concentration were due to end product inhibition which was confirmed by the experiment using the medium containing 10 gl^{-1} initial lactate (Ishizaki and Ohta, 1989).

Determination of type of inhibition

The specific growth rate and the specific glucose consumption rate are plotted according to the Hannes-Woolf plots. The μ and the μ_S at L = 0 (no inhibitor level) are plotted and the values μ and μ_S at lactate in broth 5 gl^{-1} were calculated and plotted to make the graph with the parameters of different inhibitor concentrations (Fig. 1). The results showed that the kinetics of this fermentation can be expressed by uncompetitive inhibition for L-lactate.

Specific growth rate

The equation for uncompetitive inhibition for specific growth rate is given by :

$$\mu = \frac{\mu_{max}(S_b)}{(Km)_\mu + \{1+(Ki)_\mu(L_b)\}(S_b)} \tag{5}$$

and when the value of $(Km)\mu$ is very small, this equation can be approximated to the following equation.

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{(Ki)_\mu}{\mu_{max}}(L_b) \tag{6}$$

Since the value of $(Km)\mu$ was found to be very small, the specific growth rate of this microorganism should be expressed by Eq. 6. The specific growth rate shown in Tables 1-3 were plotted against L-lactate concentration and Fig. 2 confirmed the relationship given by Eq. 6 with the values of $\mu_{max} = 1.25h^{-1}$ and $(Ki)\mu = 0.2lg^{-1}$ according to the graph at concentration of lactate in broth lower than $10gl^{-1}$.

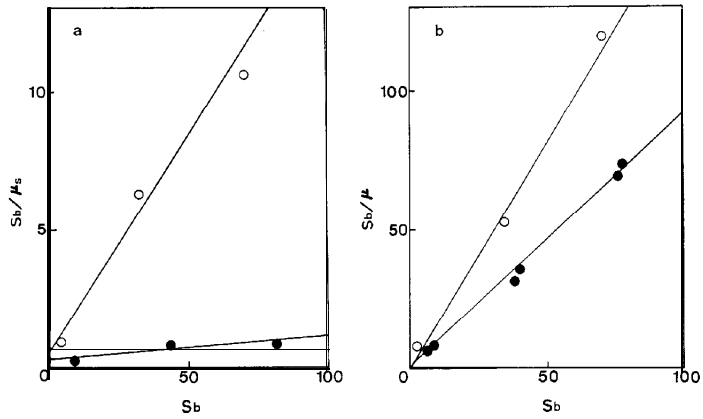

Fig. 1. Hannes-Woolf plots. a, S_b vs. S_b/μ_s; b, S_b vs. S_b/μ. Symbols (●) and (○) indicate lactic acid concentration negligibly low and 5 g l^{-1} respectively.

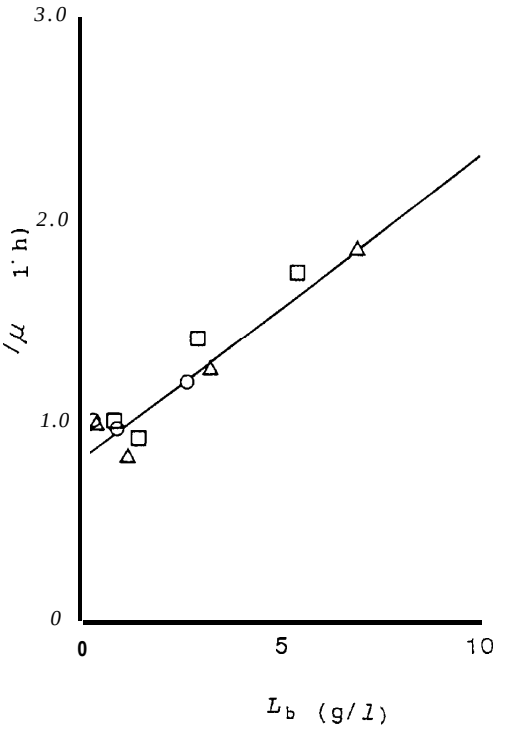

Fig. 2. The plot of specific growth rate and L-lactate concentration according to Eq. (2). Results obtained from the fermentation of $S_0 = 10\ g l^{-1}$ (○), $50\ g l^{-1}$ (△) and $80\ g l^{-1}$ (□).

Therefore specific growth rate of *L. lactis IO-1* could be written in the following formula.

$$\mu = \frac{1.25}{1+0.2(L_b)} \tag{7}$$

However, the linearity of this graph was kept only at low lactate concentration and as shown in Fig. 3, the linearity was lost at high lactate concentrations. This might be due to the formation of sterile cells but this matter will be discussed in detail later.

Specific substrate consumption rate

Specific substrate consumption rate is also expressed by uncompetitive inhibition.

$$\mu_S = \frac{(\mu_S)_{max}(S_b)}{(Km)_S + \{1+(Ki)_S(L_b)\}(S_b)} \tag{8}$$

Above equation can be reformed as,

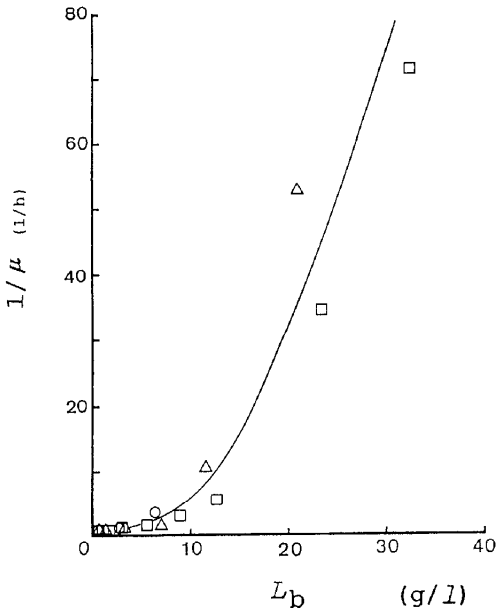

Fig. 3. The plot of specific growth rate and L-lactate concentration at high lactate concentration. Symbols are the same as in Fig. 2.

$$\frac{1}{\mu_S} = \frac{1}{(\mu_S)_{max}}\{1+\frac{(Km)_S}{S_b}\}+\frac{(Ki)_S}{(\mu_S)_{max}}(L_b) \tag{9}$$

When inhibitor concentration is very low, Eq. 9 can be approximated to the following formula (Eadie plot).

$$\mu_S = (\mu_S)_{max}-(Km)_S\frac{\mu_S}{(S_b)} \tag{10}$$

Data shown in Tables 1-3 were selected to indicate the specific substrate consumption rate at very low lactate concentrations. The results are shown in Table 4 and Fig. 4 is an Eadie plot of the data. The vertical axis indicates the value of $(\mu_S)_{max}=110h^{-1}$

Table 4. Specific substrate consumption rate at very low lactate concentration

Time h	μ_s h^{-1}	S_b gl^{-1}	μ_s/S_b $lg^{-1}h^{-1}$
2	30.70	9.69	3.17
3.5	58.92	45.67	1.29
2.5	85.50	82.93	1.00

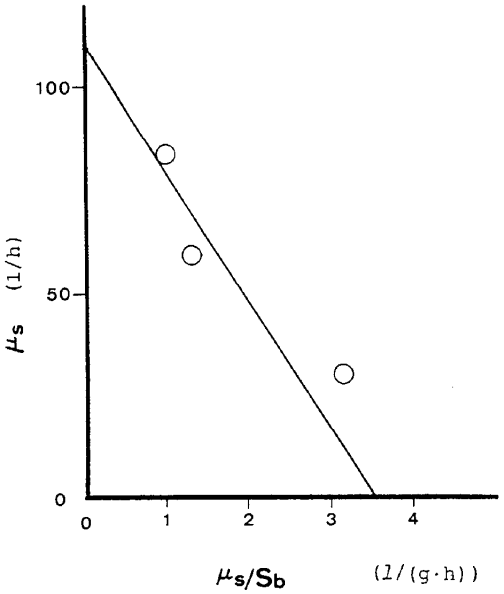

Fig. 4. Eadie plot of the data shown in Table 4.

and the slope indicates the value $(Km)_s$ 30gl^{-1}. Equation 8 is rearranged as ;

$$\frac{S_b}{\mu_s} = \frac{(Km)_s}{(\mu_s)_{max}} + \frac{1+(Ki)_s(L_b)}{(\mu_s)_{max}}(S_b) \tag{11}$$

and the term $(Ki)_s$ can be found from the slope of the plot S_b/μ_s vs. S_b. Data from Table l-3 were processed to obtain the specific substrate consumption rates with the substrate concentrations at various lactate levels. Table 5 shows the data and Fig. 5 is the plot of S_b/μ_s vs. S_b. The slope of both plots indicates a $(Ki)_s$ value 4.0 lg^{-1}. Thus the specific substrate consumption rate of *L. lactis* IO-1 can be expressed by the uncompetitive inhibition formula as follows.

Table 5. Specific substrate consumption rate at different lactate concentration

Lactate level	Time h	μ_s h^{-1}	S_b gl^{-1}	S_b/μ_s ghl^{-1}
L_b = 5.0 gl^{-1}	7.44	3.65	3.90	1.07
	10.06	5.51	37.46	6.80
	8.35	7.08	75.97	10.74
L_b = 20.0 gl^{-1}	16.05	1.65	17.53	10.62
	13.85	1.13	54.16	47.94

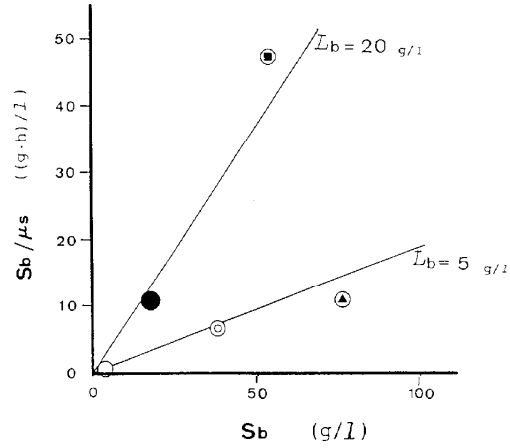

Fig. 5. The plot of S_b/μ_s vs. substrate concentration in broth with the parameter of lactate concentraion. Results obtained from the fermentation of $S_0 = 10\,g\,l^{-1}$(○), 50 $g\,l^{-1}$(● ◎) and 80 $g\,l^{-1}$(■ ▲).

$$\mu_S = 30 + \{1 + 4(L_b)\}(S_b) \qquad (12)$$

Specific lactate production rate

Since the substrate consumption rate is regulated by L-lactate concentration, the lactate production rate should also obey in the same mode. Lactate production can be expressed by the substrate consumption multiplied by a yield factor.

$$dL = -dS \cdot d\,Y_{L/S} \qquad (13)$$

The above equation is rearranged and both the numerator and denominator are divided by Xdt and then Eq. 13 is reformed as :

Table 6. Data selected from Table 1 3 to calculate the lactate formation kinetic constants

S_b $g\,l^{-1}$	L_b $g\,l^{-1}$	$Y_{L/S}$	Note
8.62	0.58	0.812	from Table 1
34.61	7.16	0.927	from Table 2
13.15	39.91	0.984	from Table 3

$$d\,Y_{L/S} = -\frac{\frac{dL}{X\,dt}}{\frac{dS}{X\,dt}} = \frac{\mu_L}{\mu_S} \tag{14}$$

Thus the differential yield factor must also be expressed by the function of uncompetitive inhibition enzyme kinetics.

$$d\,Y_{L/S} = \frac{(\mu_L)_{max}[(Km)_S + \{1+(Ki)_S(L_b)\}(S_b)]}{(\mu_S)_{max}[(Km)_L + \{1+(Ki)_L(L_b)\}(S_b)]} \tag{15}$$

One datum was selected from each table from Table 1 to 3 to compose the three sequential equations described in the Appendix section. Selected data are given in Table 6. The three unknowns were determined as $(\mu_L)_{max} = 31.5 l h^{-1}$, $(Km)_L = 6.3\ g l^{-1}$ and $(Ki)_L = 1.15\ l g^{-1}$ respectively. Thus the specific lactate production rate can be expressed as :

$$\mu_L = \frac{31.5(S_b)}{6.3 + \{1+1.15(L_b)\}(S_b)} \tag{16}$$

and the differential yield factor can be written as follows.

$$d\,Y_{L/S} = -\frac{31.5[30 + \{1+4(L_b)\}(S_b)]}{110[6.3 + \{1+1.15(L_b)\}(S_b)]} \tag{17}$$

Mathematical modeling

It was found that the fermentation kinetics of *L. lactis* IO-1 was expressed by uncompetitive inhibition of L-lactate. Hence the mathematical model can be constructed by the formulae with the determined values for constants and it is possible to simulate the fermentation time course of this bacteria by computer program. The calculation starts by giving the initial conditions i. e. inoculum size, initial sugar concentration, initial inhibitor concentration and lag time. After lag time, the growth starts and cell concentration after the shortest time dt is expressed by the following equation.

$$X = X_o \exp(\mu\,dt) \tag{18}$$

However, as stated in the beginning part of this text, at above a certain lactate concentration, μ must be affected by another factor than lactate concentration. We predicted that this might be due to formation of sterile cells which no longer grow. To study this matter, Eq. 18 was modified as,

$$X = X_o(1-\alpha)\exp(\mu\,dt) \tag{19}$$

where a is defined as "the ratio of sterile cells formed to total cell population measured by 0. D. for time dt."

In previous publications, death of the cells was expressed by the term of the specific death rate k (Pirt, 1985),

$$\mu' = p - k \tag{20}$$

where μ' is an apparent specific growth rate and the relationship between k and α can

be written by the following equation.

$$k = \frac{1}{\mathrm{d}t} \ln \frac{1}{1-\alpha} \tag{21}$$

Anyway, the cell mass concentration after the individual passed shortest time $\mathrm{d}t$ can be given by Eq. 19. When the cell concentration X at the time $t+\mathrm{d}t$ is measured, the sugar consumption during the time $\mathrm{d}t$ can be calculated by :

$$-\mathrm{d}S = \mu_S X \mathrm{d}t \tag{22}$$

therefore residual sugar concentration S is given by the following formula.

$$\mathrm{S} = S_0 + \mathrm{d}S = S - \mu_S X \mathrm{d}t \tag{23}$$

Lactate production is given by substituting $\mathrm{d}S$ into Eq. 13 and the lactate concentration is calculated as follows,

$$L = L_0 + \mathrm{d}L = L_0 - \frac{\mathrm{d}S \mu_L}{\mu_S} \tag{24}$$

The sugar concentration and lactate concentration in broth will be estimated by multiplying the concentration calculated above by the dilution rate expressing the

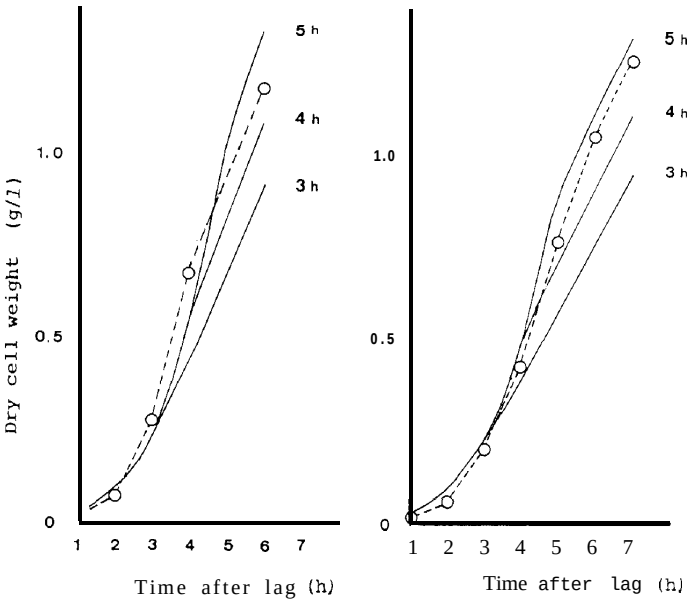

Fig. 6. Comparison between the observed results and computer simulation with the different time for the commencement of sterile cell formation with $\alpha = 0.0022$. The figures in the graph indicate the time in hour which lasts the cell growth without sterile cell formation after lag time. The left figure shows the fermentation of $S_0 =$ 10 $g l^{-1}$ and the right is $S_0 = 85 g l^{-1}$. The solid line indicates the simulation results and symbol (○) with the broken line indicates the observed results.

volume change during the culture.

$$S_b = S\frac{V}{V+\mathrm{d}V} \tag{25}$$

$$L_b = L\frac{V}{V+\mathrm{d}V} \tag{26}$$

As far as residual sugar concentration is above 0, the calculation will continue to work and give a solution for individual concentrations. The specific rates μ,μ_S and μ_L vary with time according to the substrate concentration and inhibitor concentration changes, so that the solution for the equations stated above can only be obtained by high spped numerical calculation by computer (Prosser, 1989).

The ratio of sterile cells formed to total cell population

By the mathematical model stated above, computer simulation of the cell qrowth of *L. lactis* I0-1 was carried out. Comparison between computer simulation and observed results was done to study the influence of time difference of the commencement of sterile cell formation on cell growth. Figure 6 shows that simulation with 5 h after lag time for the commencement time gave satisfactory approximation to the observed results. Figure 7 shows the relationship between the α value and the maximum viable cell concentration. At α = 0.0022 for dt = 0.01 h, estimated maximum viable cell concentration approximately agreed with the observed cell concentration measured by optical absorbance (0. D.) (dry cell weight). In all fermentations with different initial sugar concentrations except $S_0 = 10 g l^{-1}$, the maximum cell concentration reached the same value. From this fact, it is assumed that the time for the growth

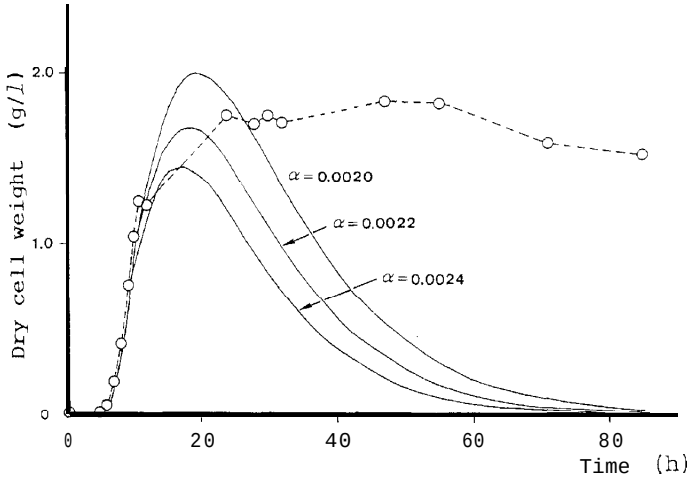

Fig. 7. Observed dried cell weight determined by optical absorbance and computer simulation at the different α for $S_0 = 85 g l^{-1}$. Lag time for simulation is 3 h in all cases.

Table 7. Approximate apparent specific growth rate ($\frac{1}{t}\ln\frac{X_t}{X_0}$) determined by viable cell count and apparent specific growth rate (μ -k) calculated from lactate concentration and $\alpha = 0.0022$

Time h	Viable Cell Cells	$\frac{1}{t}\ln\frac{X_t}{X_0}$ $m l^{-1}$ h^{-1}	lactate $g l^{-1}$	μ h^{-1}	$\mu - k$ h^{-1}
24	4.2×10^9		28.8	0.185	-0.035
60	2.0×10^8	−0.085	42.1	0.133	−0.087
84	2.9×10^7	-0.115	43.7	0.128	−0.092

with $\alpha = 0$ is always the same and α is constant during the culture. Since the dry cell weight measured by 0. D., it must include sterile cells. This makes the big difference between the computer simulation and fermentation results. To study this subject, the viable cell number in culture broth were counted. The apparent specific growth rate μ-k is approximately determined by :

$$\mu - k = \frac{1}{t}\ln\frac{X_t}{X_0} \tag{27}$$

The estimated value of μ-k by substituting viable cell count in Eq. 27 was compared with that calculated for p-k substituting lactate concentration in broth in Eq. 7 with $\alpha = 0.0022$. As shown in Table 7, both data agreed fairly good.

Enzyme inactivation scheme

The computer simulation at different initial sugar concentrations from 10 $g l^{-1}$ to 80 $g l^{-1}$ were compared with the fermentation results. In lower initial sugar levels,

Table 8. Results with sampling time for the fermentation of 60 $g l^{-1}$ initial glucose

Time h	Turbid cell mass $g l^{-1}$	Residual glucose $g l^{-1}$	Lactate $g l^{-1}$	LDH $IU l^{-1}$	Viable cell $CFU l^{-1}$	Viable cell DCW $g l^{-1}$
0	0.006	59.6(100)		0.8		
4	0.03	58.5(98.2)	0.2			
6	0.06	57.1(95.9)	1.7	9.7		
8	0.29					
10	1.02	46.5(78.1)	18.2	175.4		
12	1.51			280.2	55×10^8	1.5
16	1.67	30.7(51.5)	28.7	294.9		
19	1.68	25.2(42.2)	32.2			
21	1.71	24.1(40.5)	34.1			
24	1.71	21.1(35.3)	36.6	206.3	48×10^8	1.32
28	1.75	16.5(27.7)	40.2			
35	1.75	10.8(18.0)	44.3	147.6		
36					28×10^8	0.77
43	1.70	5.5(9.2)	48.9			
48				87.5	9x108	0.25
52	1.69	0.4(0.6)	53.7			

Table 9. LDH activity increasing ration in contrast with cell mass concentration increasing ratio at lag and exponential growth phase

Time h	Cell mass		LDH	
	Conc. gl^{-1}	Increasing ratio	Activity IUl^{-1}	Increasing ratio
0	0.006		0.8	
6	0.06	10	9.7	12
10	1.02	17	175.4	18
16	1.67	1.6	294.9	1.7

Turbid cell mass concentration is approximated to viable cell mass concentration at the viable cell increasing phase.

computer simulation and fermentation results agreed satisfactorily, however, at the end phase of the culture with high initial sugar concentration, the simulation fo sugar consumption and product formation have a tendency to be smaller than the observed results. This is suspected that the fermentation rate such as sugar consumption rate and product formation rate might be affected by the enzyme inactivation other than sterile cell formation. LDH activity changes during fermentation in contrast with other fermentation parameters i. e. dry cell weight, viable cell concentration converted from the CFU, residual glucose % and L-lactate concentration are presented in Table 8. This introduced the fact that the enzyme inactivation rate is smaller than the sterile cell formation rate. As shown in Table 9, the LDH activity increase in proportion to the viable cell concentration at the viable cell concentration increasing phase, however, after the viable cell concentration reached to a maximum, LDH activity decreased with the constant rate as the viable cell concentration decreased. A fine relationship between log LDH and culture time was found in Fig. 8 Thus, the LDH, the key enzyme

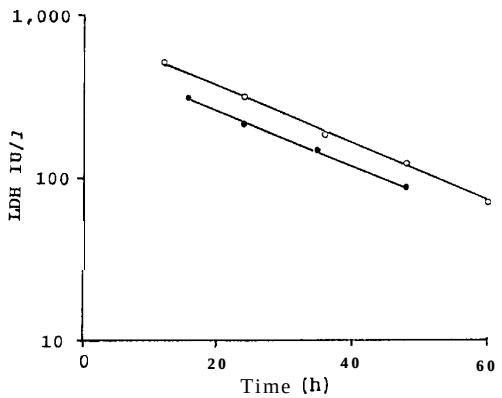

Fig. 8. Semi-log plot of LDH at the viable cell decreasing phase vs. fermentation time. Symbols; (○) LDH for the culture 70 gl^{-1} initial glucose and (●) LDH for the culture 60 gl^{-1} initial glucose.

for this fermentation, lost its activities at a constant rate when the viable cell concentration decreased. The enzyme inactivation scheme to express the enzyme activity change during fermentation was therefore indicated as ;

(i) In the viable cell concentration increasing phase, enzyme activity for fermentation can be expresed in the function of viable cell concentration.

(ii) In the viable cell concentration decreasing phase, enzyme activity decreases with a constant rate in the exponential relationship. This relationship can be written as,

$$E = E_0 \exp\{-0.036 \times (t_0 \quad t)\} \tag{28}$$

where E_0 is enzyme activity at time t_0 (h) when the viable cell concentration reaches a maximum and E gives enzyme activity at time t (h). It is suspected that there are many reasons for the LDH activity loss including autolysis.

Computer simulation for L-lactate batch fermentation employing *L. lactis* 10-1

The computer simulation program for L-lactate batch fermentation using *L. lactis* IO-1 in which sterile cell formation rate and enzyme inactivation rate were built has completed and the flow chart for the program is shown in Fig. 9. To prove the reliability of this simulation program, the calculation results for 60 $g l^{-1}$ initial glucose and 0.01 $g l^{-1}$ inoculum size as initial condition were given by the CRT hard copy chart of the computer in contrast with the observed results (Fig. 10). Figure 11 shows the fermentation results for the culture of 10 $g l^{-1}$ initial glucose and 0.009 $g l^{-1}$ inoculum size, which are demonstrated over the CRT hard copy of the computer simulation started at the same initial glucose concentration and the inoculum size. Both cases gave a satisfactory approximation.

Thus, L-lactate batch fermentation using *L. lactis* IO-1 can be simulated accurately and the calculation results agreed almost perfectly with the fermentation results for the whole culture phase.

Appendix

Kinetic parameters in Eq. (15) can be determined by the following procedure. Since the constants for the equation of specific substrate consumption rate were already determined, there are three remained unknowns and those unknowns can be solved by the following three sequetial equations.

$$(\mathrm{d}Y_{L/S}) = \frac{(\mu_L)_{max}[30+\{1+4(L_b)_1\}(S_b)_1]}{110[(Km)_L+\{1+(Ki)_L(L_b)_1\}(S_b)_1]} \tag{A-1}$$

$$(\mathrm{d}Y_{L/S}) = \frac{(\mu_L)_{max}[30+\{1+4(L_b)_2\}(S_b)_2]}{110[(Km)_L+\{1+(Ki)_L(L_b)_2\}(S_b)_2]} \tag{A-2}$$

$$(\mathrm{d}Y_{L/S}) = \frac{(\mu_L)_{max}[30+\{1+4(L_b)_3\}(S_b)_3]}{110[(Km)_L+\{1+(Ki)_L(L_b)_3\}(S_b)_3]} \tag{A-3}$$

Three equations are combined and rearranged, and then the three unknowns are given as follows :

$$(Ki)_{\mathrm{L}} = \frac{(J_1-J_2)(K_3-K_4)-(J_3-J_4)(K_1-K_2)}{(J_3-J_4)\{(L_{\mathrm{b}})_1K_1-(L_{\mathrm{b}})_2K_2\}-(J_1-J_2)\{(L_{\mathrm{b}})_2K_3-(L_{\mathrm{b}})_3K_4\}} \quad \textbf{(A-4)}$$

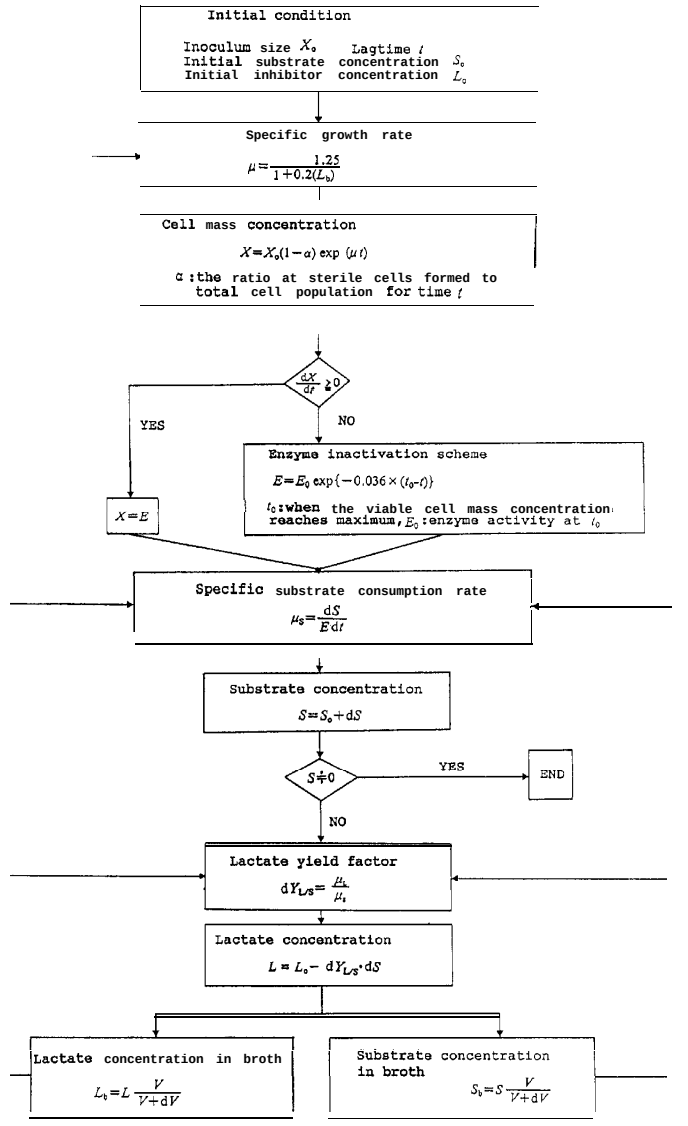

Fig. 9. The flow chart for computer simulation program applying enzyme inactivation scheme.

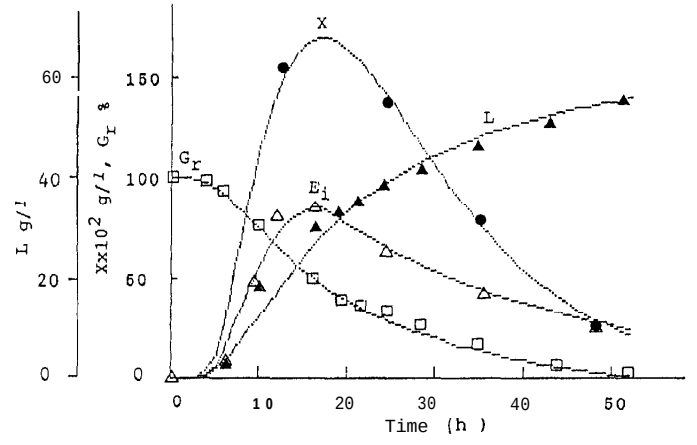

Fig. 10. CRT hard copy of computer simulation started at initial glucose 60 $g l^{-1}$ and inoculum size 0.01 $g l^{-1}$ and symbols, (●) viable cell DCW converted from CFU, g l^{-1}, (□) residual glucose, %, (A) L-lactate produced, g l^{-1}, (△) LDH activity, IU l^{-1}, indicate the observed results of the fermentation with initial conditions the same as those for computer simulation. Abbreviations : X is the viable cell concentration in DCW, g l^{-1}, G_r is residual glucose, %, L is L-lactate concentration, $g l^{-1}$ and E_i is LDH activity index indicating the ratio of LDH over $LDH_{max}(E_0)$ which is given at the time for maximum viable cell concentration.

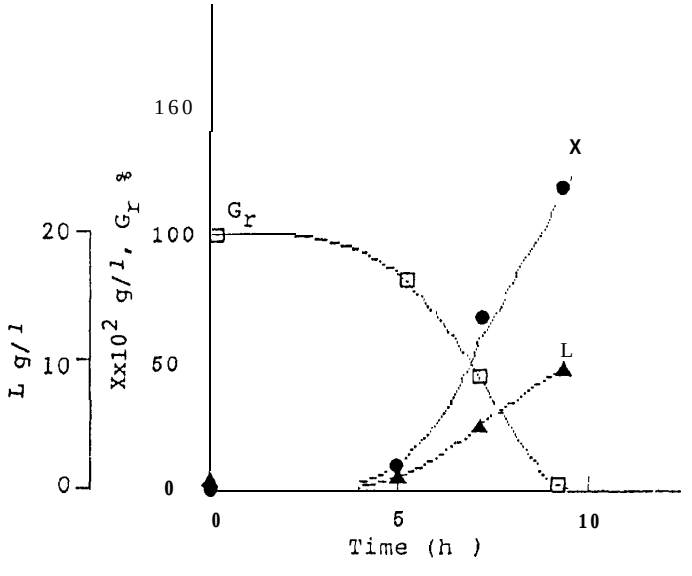

Fig. 11. CRT hard copy of computer simulation in contrast with fermentation results started at initial glucose 10 g l^{-1} and inoculum size 0.01 $g l^{-1}$. Abbreviations and symbols are same as Fig. 10.

$$(Km)_L = \frac{\{1+(Ki)_L(L_b)_1\}K_1 - \{1+(Ki)_L(L_b)_2\}K_2}{J_1 - J_2} \quad \text{(A-5)}$$

$$(\mu_L)_{max} = \frac{110(dY_{L/S})_1[(Km)_L + \{1+(Ki)_L(L_b)_1\}(S_b)_1]}{30+\{1+4(L_b)_1\}(S_b)_1} \quad \text{(A-6)}$$

where

$$J_1 = (dY_{L/S})_2[30+\{1+4(L_b)_1\}(S_b)_1] \quad \text{(A-7)}$$

$$J_2 = (dY_{L/S})_1[30+\{1+4(L_b)_2\}(S_b)_2] \quad \text{(A-8)}$$

$$J_3 = (dY_{L/S})_3[30+\{1+4(L_b)_2\}(S_b)_2] \quad \text{(A-9)}$$

$$J_4 = (dY_{L/S})_2[30+\{1+4(L_b)_3\}(S_b)_3] \quad \text{(A-10)}$$

$$K_1 = (S_b)_1 J_2 \quad \text{(A-11)}$$

$$K_2 = (S_b)_2 J_1 \quad \text{(A-12)}$$

$$K_3 = (S_b)_2 J_4 \quad \text{(A-13)}$$

$$K_4 = (S_b)_3 J_3 \quad \text{(A-14)}$$

Nomenclature

dt = the shortest time passed, h.
$dY_{L/S}$ = the differential yield factor.
E = enzyme activity.
k = the specific death rate, h^{-1}
Ki = reciprocal of the inhibitor constant for specific rate, lg^{-1}.
Km = Michaelis comstant. gl^{-1}.
L = L-lactate concentration, gl^{-1}.
S = sugar concentration, gl^{-1}.
t = time, h.
V = hypothetical volume to determine the concentration X, S and L, l.
dV = volume change during dt due to operation, l.
X = cell mass concentration, gl^{-1}
α = the ratio of sterile cell formed for time dt.
μ = specific growth rate, h^{-1}.
μ' = apparent specific growth rate, h^{-1}.
μ_S = specific substrate consumption rate, h^{-1}.
μ_L = specific product formation rate, h^{-1}.

< Subscripts >
b = concentration in broth.
0 = initial concentrations, initial time.

1, 2, 3 = the number to specify the combination of the data, the differential yield factor, substrate concentration and lactate concentration, to solve the three sequential equations for lactate formation kinetics.
μ = parameter for specific growth rate.
S = parameter for specific substrate consumption rate.
L = parameter for specific lactate formaion rate.
max = maximun value.

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