Kinetics of Biphasic Growth of Yeast in Continuous and Fed-Batch Cultures

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Summary

The influence of dilution rate on the production of biomass, ethanol, and invertase in an aerobic culture of *Saccharomyces carlsbergensis* was studied in a glucose-limited chemostat culture. A kinetic model was developed to analyze the biphasic growth of yeast on both the glucose remaining and the ethanol produced in the culture. The model assumes a double effect where glucose regulates the flux of glucose catabolism (respiration and aerobic fermentation) and the ethanol utilization in yeast cells. The model could successfully demonstrate the experimental results of a chemostat culture featuring the monotonic decrease of biomass concentration with an increase of dilution rate higher than 0.2 hr^{-1} as well as the maximum ethanol concentration at a particular dilution rate around 0.5 hr^{-1}. Some supplementary data were collected from an ethanol-limited aerobic chemostat culture and a glucose-limited anaerobic chemostat culture to use in the model calculation. Some parametric constants of cell growth, ethanol production, and invertase formation were determined in batch cultures under aerobic and anaerobic states as summarized in a table in comparison with the chemostat data. Using the constants, a prediction of the optimal control of a glucose fed-batch yeast culture was conducted in connection with an experiment for harvesting a high yield of yeast cells with high invertase activity.

INTRODUCTION

The Crabtree effect has been known as a phenomenon of stimulated fermentation metabolism in aerobic culture of some yeast strains in the presence of a sufficient amount of glucose. This effect was observed most remarkably in the growth of *Saccharomyces carlsbergensis*.[1] In batch culture, the glucose concentration is comparatively high in the first growth period, so that ethanol fermentation takes place inevitably despite the presence of sufficient dissolved oxygen. Ethanol secreted so far is assimilated by yeast in the later growth phase.[2] If the glucose concentration in the culture is maintained at low concentrations during growth by means of fed-batch culture, glucose flux to fermentative metabolism may become

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so small that the yeast growth yield is improved to a certain extent since respiration is evidently more efficient in supplying biosynthetic energy for biomass production than fermentation.

A kinetic study of yeast growth particularly at low glucose concentrations is essential for the design of rational control of the feeding rate of glucose. Following the previous work on growth kinetics of yeast in a batch culture, the yeast growth rate at low glucose concentrations was investigated in a chemostat culture in this work to further understand the quantitative aspect of biphasic growth. Von Meyenburg[3] has examined the dilution rate effect on yeast growth in a glucose-limited aerobic chemostat culture, establishing an experimental result similar to that obtained in this study. Von Meyenburg's result has been analyzed by Bijkerk and Hall,[4] assuming a two-stage growth model (metabolizing cells and reproducing cells) to simulate the variation in the concentrations of biomass, glucose, and ethanol with dilution rate in a chemostat culture. Bijkerk and Hall suggested that their model was satisfactory in predicting the biomass concentration but not the ethanol concentration. Van Dedem and Moo-Young[5] reported a model calculation of biphasic growth on two carbon substrates assuming induction and catabolite repression of a permease for the second substrate. In this work, biphasic growth was analyzed from another standpoint considering regulatory action of glucose upon both utilization of ethanol and glucose catabolism.

THEORY

The following assumptions are made in analyzing the growth of yeast utilizing glucose as a carbon source in an aerobic chemostat culture:

1) Glucose transported into the cells is catabolized by both respiratory and fermentative metabolisms. The fraction of fermented glucose in total glucose absorbed [$R(S)$] varies depending on the glucose concentration (S) in the culture.

2) Ethanol accumulated in the culture by fermentative catabolism can be reutilized by yeast. The utilization rate is repressed to a factor of $Q(S)$, if glucose (S) is utilized simultaneously as another carbon source.

3) All stoichiometric coefficients: growth yields on respired glucose ($Y_{X/S}^{resp}$), respired ethanol ($Y_{X/A}^{resp}$), and fermented glucose ($Y_{X/S}^{ferm}$), as well as ethanol yield on fermented glucose ($Y_{A/S}^{ferm}$), are constant irrespective of growth rate.

4) The three metabolisms in yeast cells, respiration on glucose, and respiration on ethanol and fermentation on glucose, are quantitatively additive.

5) Maintenance metabolism is neglected in comparison to growth metabolism.

Referring to the simple scheme of glucose metabolism in yeast considered above, a mass balance of biomass ($\bar{X}$), glucose ($\bar{S}$), and ethanol ($\bar{A}$) in a chemostat culture at dilution rate D gives eqs. (1)–(3), following the growth kinetics of Monod:

$$D\cdot\bar{X} = [\mu_S{}^{\max}\cdot\bar{S}/(K_S + \bar{S})]\bar{X} + Q(\bar{S}) \cdot[\mu_A{}^{\max}\cdot\bar{A}/(K_A + \bar{A})]\cdot\bar{X} \quad (1)$$

$$= G_S + G_A \quad (1')$$

$$D\cdot(S_R - \bar{S}) = G_S/\{[1 - R(\bar{S})]\cdot Y_{X/S}^{\text{resp}} + R(\bar{S})\cdot Y_{X/S}^{\text{ferm}}\} \quad (2)$$

$$D\cdot\bar{A} = Y_{A/S}^{\text{ferm}}\cdot D\cdot(S_R - \bar{S})\cdot R(\bar{S}) - (1/Y_{X/A}^{\text{resp}})\cdot G_A - K_L a\cdot\bar{A} \quad (3)$$

where S_R is the glucose concentration in a reservoir vessel and $K_L a$ is a volumetric mass transfer coefficient of ethanol evaporation from culture liquid. From eqs. (1'), (2), and (3), $R(\bar{S})$ correlates with $\bar{X}$, $\bar{A}$, and $\bar{S}$ as follows:

$$R(\bar{S}) = \frac{Y_{X/S}^{\text{resp}}(S_R - \bar{S}) - \bar{X} - Y_{X/A}^{\text{resp}}(1 + K_L a/D)\cdot\bar{A}}{(Y_{X/S}^{\text{resp}} - Y_{X/S}^{\text{ferm}} - Y_{X/A}^{\text{resp}}\cdot Y_{A/S}^{\text{ferm}})(S_R - \bar{S})} \quad (4)$$

The stoichiometric and kinetic parameters in eqs. (1), (2), and (3) can be determined from the experimental data of glucose- and ethanol-limited chemostat cultures under aerobic conditions and glucose-limited chemostat culture under anaerobic conditions, as will be carried out later.

MATERIALS AND METHODS

Organism

The organism used was *Saccharomyces carlsbergensis* strain No. LAM 1068.

Medium

Table I shows the medium composition. For the procedure used to prepare the medium, see the previous report.[2]

TABLE I
Medium Composition (Per liter Distilled Water)

	Amount
Glucose	1.0 g
NH_4Cl	2.0 g
KH_2PO_4	88 mg (20 mg as P)
Potassium citrate, monobasic	2.3 g
NaOH	0.4 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaCl_2$	0.3 g
KCl	0.1 g
Inositol	60 mg
DL-Asparagine	50 mg
Thiamine hydrochloride	6 mg
Pyridoxin hydrochloride	1 mg
Calcium pantothenate	0.5 mg
Biotin	0.15 mg
Citric acid·H_2O	0.5 mg
$ZnSO_4 \cdot 7H_2O$	0.5 mg
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.1 mg
$CuSO_4 \cdot 5H_2O$	0.025 mg
$MnSO_4 \cdot H_2O$	0.005 mg
H_3BO_3	0.005 mg
$Na_2MoO_4 \cdot 2H_2O$	0.005 mg
Streptomycin sulfate	0.1 g
Adekanol LG 126 (antifoamer)	0.01 ml
pH	4.6

Culture

A cylindrical glass vessel with a side pipe for overflowing liquid outlet, of which the working volume ranged from 100 to 350 ml, was used as a chemostat fermentor. The agitation and oxygen absorption of culture broth were done by dispersing sterile air bubbles into the vessel at an air flow rate of 1v/v/m through a perforated glass filter (ball type, 20 mm in diam) located at the bottom. The vessel (hereafter referred to as an aeration column) was immersed in a water bath to maintain the liquid temperature at 30°C. After one or two days of batch cultivation, sterile fresh medium was fed to the aeration column by a peristaltic pump at a constant flow rate of from 14 to 55 ml/hr. With the use of this apparatus, yeast cells growing at a steady state were harvested after two or three days of continuous culture depending on the dilution rate which ranged from 0.040 to 0.53 hr^{-1}.

Small-scale jar fermentors (2 liter) (Marubishi, MD 250) were

used for batch and fed-batch cultures under aerobic conditions as well as for an anaerobic chemostat culture. Anaerobic culture was carried out under nitrogen gas by flowing sterile N_2 gas at a flow rate of 20 ml/min through the head space of a jar fermentor.

The maximum oxygen demand of the aerobic yeast culture was measured as 107 mg O_2/liter hr, which required a volumetric oxygen transfer coefficient of 0.30 min^{-1} to maintain the dissolved oxygen concentration in the culture above a critical value of 1 ppm. The volumetric oxygen transfer coefficient (K_La) for the jar fermentor was determined as 0.83 min^{-1} at 400 ppm and 0.33 v/v/m using the sulfite oxidation method, 0.71 min^{-1} with the dynamic method[6] and 0.56 min^{-1} with the mass balance method assuming a pseudo-steady state.[6] K_La in the aeration columns was determined in the range of 0.75 to 2.81 min^{-1} with sulfite oxidation method. Therefore, it is fully expected that the oxygen transfer capacity of both the jar fermentor and the aeration column was enough for yeast respiration.

The evaporation rate of ethanol from the culture vessel was measured as the volumetric coefficient of ethanl desorption (K_La). K_La for ethanol evaporation was 0.0081-0.0246 hr^{-1} in aeration columns and 0.0088 hr^{-1} in a jar fermentor.

Determinations

The steady- and unsteady-state concentrations of yeast biomass, glucose, and ethanol in the culture broth and the invertase activity in yeast cells were determined with the same procedure as described previously.[2] The sample liquid for glucose and ethanol determinations was collected by filtering culture broth in 1 sec with a membrane filter sheet, type RA (pore size = 1.2 μm).

RESULTS AND DISCUSSIONS

Glucose-Limited Aerobic Chemostat Culture

The concentrations of yeast cells, glucose, and ethanol in the culture broth of a chemostat culture of *S. carlsbergensis* were measured at steady state with varying dilution rates from 0.118 to 0.528 hr^{-1}. The relationship is shown in Figure 1, where two remarkable points are observed: the biomass concentration decreases with an increase of dilution rate higher than 0.2 hr^{-1}; the ethanol concentration has a maximum at a dilution rate of 0.5 hr^{-1}. One of the purposes of this study is to explain these characteristic features quantitatively by assuming an appropriate yeast growth model.

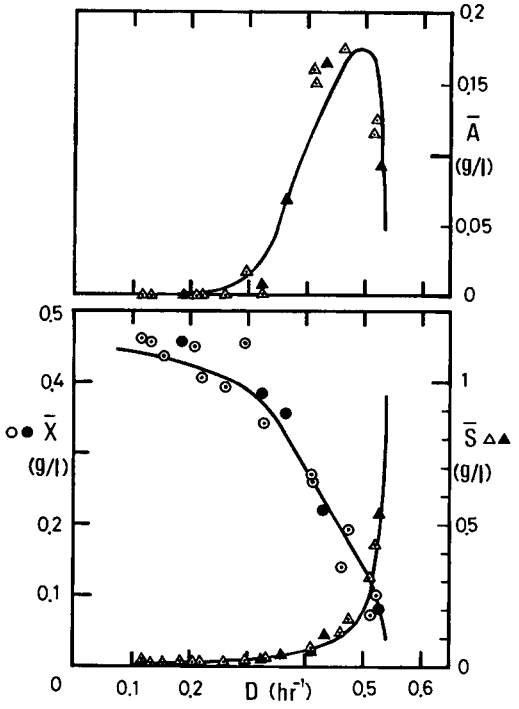

Fig. 1. Glucose-limited chemostat culture under aerobic condition. Steady-state concentration of cell mass ($\bar{X}$, ○), glucose ($\bar{S}$, △) and ethanol ($\bar{A}$, △ in upper part) are correlated to dilution rate. Open symbols denote data points used in determining parametric constants. Data after prediction study are represented by closed symbols. Solid lines for $\bar{X}$, $\bar{S}$, and $\bar{A}$ are calculations from eqs. (1)-(3), using some constants for chemostat culture shown in Table II.

A growth yield of 0.45 was determined from the biomass concentration at a dilution rate less than 0.2 hr^{-1}, where glucose was nearly depleted from the culture. This numerical value of 0.45 is assumed to be close to the growth yield on glucose by respiratory metabolism alone, i.e., $Y_{X/S}^{resp} = 0.45$.

It is interesting that the maximum ethanol concentration was observed at almost the same glucose concentration (0.1-0.2 g/liter) in batch and continuous cultures. It was assumed previously[2] in a batch yeast culture that ethanol could be utilized by yeast when the glucose concentration decreased below 0.15 g/liter. However, this assumption could not satisfactorily elucidate the experimental relationship among X, A, and D in a chemostat culture. Moreover, the ratio of glucose converted into ethanol to the total amount of glucose

absorbed by yeast [$=Q(S)$] was not constant as assumed in analyzing the growth kinetics in a batch experiment. A correlation between $Q(S)$ and S must be reevaluated that can fit for the growth subjected to substrate limitation in a chemostat culture.

Glucose-Limited Anaerobic Chemostat Culture

Two stoichiometric constants for the production of biomass and ethanol from glucose were estimated in a chemostat yeast culture under anaerobic conditions. From the results shown in Figure 2, both yields of biomass and ethanol on consumed glucose were determined as $Y_{X/S}^{\text{ferm}} = 0.14$ g cell mass/g glucose and $Y_{A/S}^{\text{ferm}} = 0.26$ g ethanol (EtOH)/g glucose, respectively. These numerical values were rather low in comparison to those determined in the medium supplemented with 0.5 g/liter peptone and 0.5 g/liter yeast extract ($Y_{X/S}^{\text{ferm}} = 0.19$ and $Y_{A/S}^{\text{ferm}} = 0.75$).

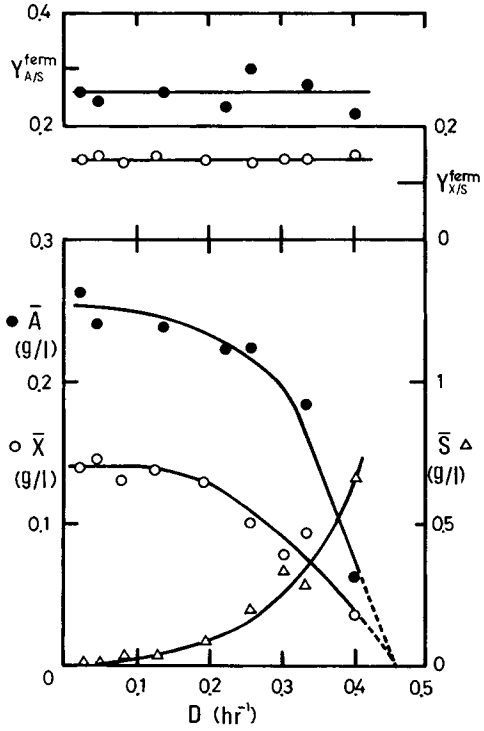

Fig. 2. Glucose-limited chemostat culture under anaerobic condition. $Y_{A/S}^{\text{ferm}} = 0.26$ and $Y_{X/S}^{\text{ferm}} = 0.14$ were estimated therefrom.

Ethanol-Limited Aerobic Chemostat Culture

By using an ethanol-containing medium as a sole carbon source instead of glucose, chemostat data were determined and plotted in Figure 3. The reciprocal plot between D and $\bar{A}$ as shown in Figure 3 gives a maximum specific growth rate of $\mu_A{}^{max} = 0.20\ hr^{-1}$ and a saturation constant of $K_A = 0.014$ g/liter. The ethanol growth yield was determined as $Y_{X/A} = 0.56$ g cells/g EtOH.

Maximum Specific Growth Rate

According to the dilution method,[7] μ_{max} of glucose-grown yeast under aerobic and anaerobic states as well as on ethanol under aerobic state were determined as 0.55, 0.46, and 0.20 hr^{-1}, respectively, from the slopes shown in Figure 4.

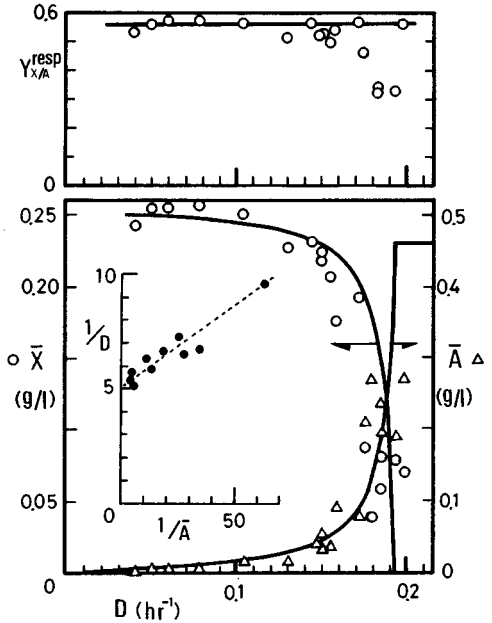

Fig. 3. Ethanol-limited chemostat culture under aerobic condition. $Y_{X/A}^{resp} = 0.56$ was determined from the result. $\mu_A{}^{max} = 0.20$ and $K_A = 0.014$ were determined from a reciprocal plot of the result as shown in the insert. Lines drawn through circles and triangles are calculations of the following equations: $\bar{X} = Y_{X/A}(A_R - \bar{A})$ and $\bar{A} = K_A \cdot D/(\mu_A{}^{max} - D)$.

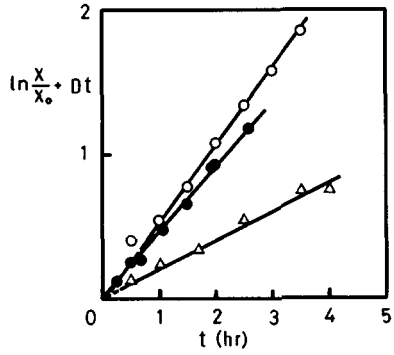

Fig. 4. Washout experiments for aerobic growth on glucose (○), anaerobic growth on glucose (●), and aerobic growth on ethanol (△). μ_{max} values for each growth rate were estimated from the slope of lines as 0.55, 0.46, and 0.20 hr^{-1}, respectively.

Procedure for Determining K_S, $Q(S)$, and $R(S)$

Although both regulation coefficients of $R(S)$ and $Q(S)$ for the aerobic fermentation of glucose and ethanol utilization in the presence of glucose were tentatively fixed in the previous study on batch culture,[2] these were reestimated in the chemostat culture. The relationship $R(S)$ vs. $\bar{S}$ was estimated from eq. (4) using 14 sets of data shown as open symbols in Figure 1 together with the predetermined constants of $Y_{X/S}^{resp} = 0.45$, $Y_{A/S}^{ferm} = 0.14$, $Y_{X/A}^{resp} = 0.56$, $K_L a$ (for ethanol evaporation) = 0.0081–0.0246 hr^{-1}, and $S_R = 1.0$ g/liter. Figure 5 shows the correlation between $R(\bar{S})$ and $\bar{S}$ thus estimated. The mathematical formulation of $R(\bar{S})$ is written as follows according to the equation developed previously for enzyme induction,[8] assuming glucose to play a role of an inductive effector for aerobic fermentation:

$$R(\bar{S}) = [1 + 1.576 \times 10^4(\bar{S})^{1.386}]/[200 + 1.576 \times 10^4(\bar{S})^{1.386}] \quad (5)$$

where a base value of $R(\bar{S})$ under full repression was arbitrarily assumed to be 0.005. The inductive effect of glucose on aerobic fermentation increases as the glucose concentration increases.

The determination of K_S was done by substituting the experimental data set of $(D, \bar{X}, \bar{S})$ into eq. (2), where $R(\bar{S})$ had been estimated in advance according to eq. (5). The numerical value of K_S was averaged as $K_S = 0.021 \pm 0.008$ g/liter from the calculations at dilution rates higher than 0.15 and lower than 0.35 hr^{-1}, where the glucose concentration was in the range of 0.008 and 0.035 g/liter.

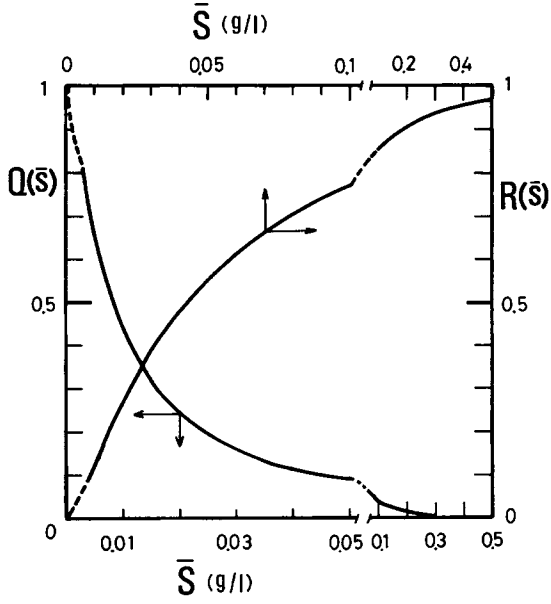

Fig. 5. Estimated correlation between $R(\bar{S})$ and $\bar{S}$ and that between $Q(\bar{S})$ and $\bar{S}$. Relationships are formulated by eqs. (5) and (6), respectively, according to induction and repression models.

Using the mean value of K_S together with the predetermined values of $\mu_S{}^{max}$, $\mu_A{}^{max}$, and K_A with the experimental data set of $(D, \bar{X}, \bar{S}, \bar{A})$, a correlation of $Q(\bar{S})$ with $\bar{S}$ was calculated from eq. (1) and is shown in Figure 5. Glucose was assumed here to function as a repressor of a key enzyme needed for utilizing ethanol as carbon source. The mathematical formulation of $Q(\bar{S})$ as written as follows according to the equation for the repression model reported in the previous paper:[8]

$$Q(\bar{S}) = [1 + 26.81(\bar{S})^{1.313}]/[1 + 536.2(\bar{S})^{1.313}] \qquad (6)$$

A low concentration of glucose of the magnitude of 0.01 g/liter can repress the utilization of ethanol by yeast.

Specific Activity of Yeast Invertase

Figure 6 shows a dependence of specific activity of invertase in yeast cells on the dilution rate of an aerobic chemostat culture. When glucose was used as a limiting carbon source in the feed medium, invertase content (E/X) increased with dilution rate up to

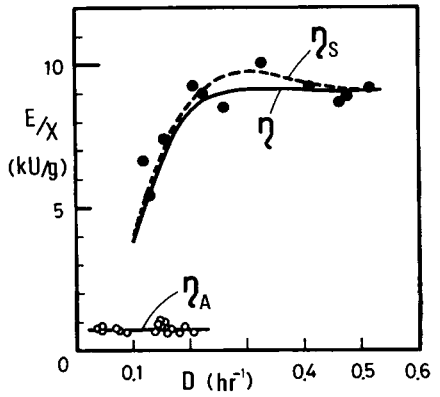

Fig. 6. Effect of dilution rate on specific invertase activity of yeast grown in glucose-limited (●) and ethanol-limited (○) chemostat cultures. (— — —) Hypothetical relationship for yeast cells utilizing glucose exclusively from a mixture of glucose and ethanol.

0.2 hr^{-1}, above which it was nearly constant. When ethanol was used instead of glucose, the specific invertase activity was almost invariable with dilution rate, although ethanol-grown cells had a very limited invertase activity in contrast to those grown on glucose. In the former culture yeast was grown on both glucose and its product, ethanol. The rate of invertase biosynthesis based on glucose consumption alone can be evaluated by subtracting invertase formation due to ethanol consumption from the above result. The ratio of invertase synthesis rate to growth rate is defined as η,

$$\eta = \frac{(dE/dt)_{\text{production}}}{(dX/dt)_{\text{growth}}} = \frac{D\bar{E}}{D\bar{X}}$$

If each η value for the yeast grown on glucose and ethanol is written as η_S and η_A, invertase content of yeast in aerobic fermentation is expressed as follows:

$$\eta = \bar{E}/\bar{X} = (\eta_S \cdot G_S + \eta_A \cdot G_A)/D\bar{X} \tag{7}$$

η_A was determined from the result shown in Figure 6 as,

$$\eta_A = 0.77 \text{ (kU/g cells hr)} \tag{8}$$

η_S values at varying dilution rates were estimated by substituting the numerical values of $\eta_A = 0.77$ and data sets of (D, $\bar{X}$, G_S, G_A) in eq. (7).

The relationship between η_S and $\bar{S}$ is written as follows according

to the equation of dual control of enzyme synthesis:[8]

$$\eta_S = 12 \cdot \frac{2950(\bar{S})^{1.500}}{1 + 2950(\bar{S})^{1.500}} \frac{1 + 421.0(\bar{S})^{1.730}}{1 + 555.3(\bar{S})^{1.730}} \tag{9}$$

The dotted line in Figure 6 shows a relationship represented by eq. (9).

Simulation of a Chemostat Culture

A prediction of some chemical changes in an aerobic chemostat culture of glucose-grown yeast was carried out using the kinetic and stoichiometric constants determined so far and the relationships of $R(\bar{S})$ and $Q(\bar{S})$ to $\bar{S}$ as shown in Figure 5. With arbitrary values of $\bar{S}$, numerical values of $\bar{A}$, $\bar{X}$, and $\bar{D}$ were calculated from the three simultaneous equations [eqs. (1)–(3)]. The results are illustrated in Figure 1 as solid curves for $\bar{X}$, $\bar{S}$, and $\bar{A}$. The closed circles in Figure 1 are another experimental result measured after the prediction was completed. The characteristic behavior of biphasic yeast growth in an aerobic chemostat culture mentioned before is reproduced clearly by the lines of model prediction.

Comparison of Batch and Chemostat Cultures

Figure 7 shows the result of batch culture in aerobic and anaerobic conditions. From anaerobic culture,

$$Y_{X/S}^{\text{ferm}} = [X(9) - X(0)]/[S(0) - S(9)]$$
$$= (0.155 - 0.0006)/(1.01 - 0) = 0.15$$

where the numbers in the parentheses indicate the time of cultivation. Similarly,

$$Y_{A/S}^{\text{ferm}} = [A(9) - A(0)]/[S(0) - S(9)] = (0.352 - 0.020)/(1.01 - 0)$$
$$= 0.33$$

From aerobic culture,

$$Y_{X/A}^{\text{resp}} = [X(14) - X(8)]/[A(8) - A(14)] = (0.410 - 0.230)/(0.268 - 0)$$
$$= 0.67$$

$$Y_{X/S} = [X(8) - X(0)]/[S(0) - S(8)] = (0.231 - 0.012)/(0.97 - 0.005)$$
$$= 0.227$$

$$Y_{A/S} = [A(8) - A(0)]/[S(0) - S(8)] = (0.268 - 0.016)/(0.97 - 0.005)$$
$$= 0.261$$

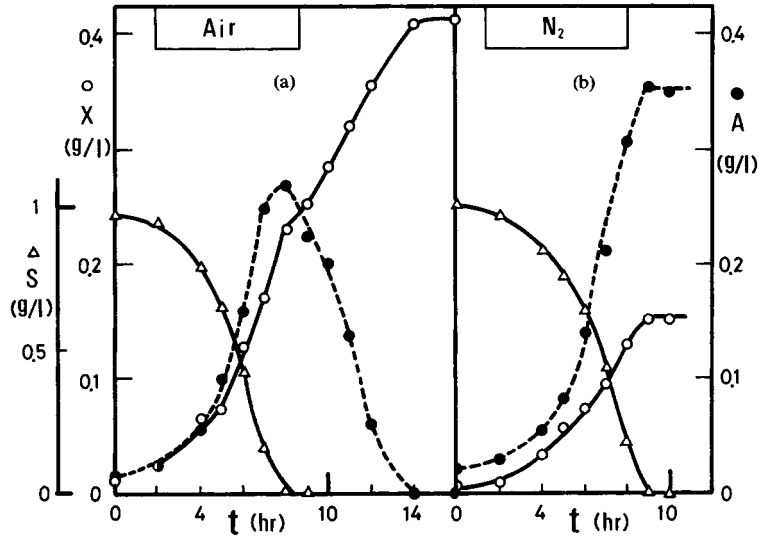

Fig. 7. Batch culture of yeast under (a) aerobic and (b) anaerobic conditions. $Y_{X/A}^{resp} = 0.52$, $Y_{X/A}^{resp} = 0.67$, $Y_{X/S}^{ferm} = 0.15$, and $Y_{A/S}^{ferm} = 0.33$ were estimated from the results.

$Y_{X/S}^{resp}$ in a batch culture was estimated as follows using some of the above constants. Equations (10) and (11) are derived by assuming the regulation coefficient of glucose catabolism, $R(S)$ varies with time in a batch culture,

$$X(t) - X(0) = \int_0^t \left(-\frac{dS}{dt}\right)_{cons} \cdot \{[1 - R(S)] \cdot Y_{X/S}^{resp} + R(S) \cdot Y_{X/S}^{ferm}\} dt \tag{10}$$

$$A(t) - A(0) = \int_0^t \left(-\frac{dS}{dt}\right)_{cons} \cdot R(S) \cdot Y_{A/S}^{ferm}\, dt \tag{11}$$

If

$$\frac{1}{S - S} \int \left(-\frac{dS}{dt}\right)_{cons} \cdot R(S)\, dt$$

is written as $\overline{R(S)}$, eqs. (10) and (11) are converted to eqs. (10′) and (11′):

$$Y_{X/S} = [X(t) - X(0)]/[S(0) - S(t)] = Y_{X/S}^{resp} \cdot [1 - \overline{R(S)}] + Y_{X/S}^{ferm} \cdot \overline{R(S)} \tag{10'}$$

$$Y_{A/S} = [A(t) - A(0)]/[S(0) - S(t)] = \overline{R(S)} \cdot Y_{A/S}^{ferm} \tag{11'}$$

By substituting $Y_{A/S}^{ferm}$ and $Y_{A/S}$ in eq. (11'), it was estimated that $R(S) = 0.79$. By substituting $Y_{X/S}^{ferm}$, $Y_{X/S}$, and $R(S) = 0.79$ in eq. (10'), it was estimated that $Y_{X/S}^{resp} = 0.52$.

The characteristic constants determined in the batch cultures were compared with those in the chemostat cultures. Table II shows a summary of the comparison. The maximum specific growth rates on glucose and ethanol are much higher in chemostat than in batch cultures. Presumably, some yeast cells with higher growth rates might be selected in chemostat cultures. This may show that a continuous culture is a better method than a batch culture for expecting rapid yeast growth. On the other hand, the numerical values of the stoichiometric coefficient are slightly higher in batch culture. For reference, some of the kinetic and stoichiometric parameters were surveyed in the literature. A $Y_{X/S}^{ferm}$ of 0.14 estimated in this work is comparable in magnitude to 0.162,[9] 0.089,[10] and 0.101,[11] in the literature. The experimental range of $Y_{A/S}^{ferm}$ of 0.26 and 0.33 is rather small compared with the literature values of 0.46[9,12] and 0.47.[10]

However, this quantity could be increased in our experiment to 0.34–0.75 in an anaerobic chemostat culture by supplying the medium with 0.1–1.0 g/liter of a mixture of peptone and yeast extract.

TABLE II
Kinetic and Stoichiometric Parameters of *S. carlsbergensis* Grown in Batch and Chemostat Cultures

Parameter	Culture	
	batch	chemostat
μ_S^{max} (hr^{-1})	0.39[a]	0.55
μ_A^{max} (hr^{-1})	0.11[a]	0.20
K_S (g/liter)	–	0.021[a]
K_A (g/liter)	–	0.014[a]
$Y_{X/S}^{resp}$ (g/g)	0.52[a]	0.45
$Y_{X/A}^{resp}$ (g/g)	0.67[a]	0.56
$Y_{X/S}^{ferm}$ (g/g)	0.15[a]	0.14
$Y_{A/S}^{ferm}$ (g/g)	0.33[a]	0.26
$R(S)$	$\overline{R(S)} = 0.79$	Fig. 6[a]
$Q(S)$	–	Fig. 6[a]

[a] Used in the calculation of chemical changes for a fed-batch culture.

The difference mentioned above ascribes to a difference of synthetic and complex media.

μ_S^{max} values of 0.39 and 0.55 in batch and chemostat cultures are slightly higher in comparison to 0.30–0.31,[11] and 0.44,[13] in the literature, although the microbial strains were different. The fraction of glucose catabolized in fermentative metabolism, $R(S)$ of 0.79 in a batch culture is in good accord with the reported value of 0.74.[11] $Y_{X/S}^{resp}$ of 0.45 and 0.52 is similar to $Y_{X/S}$ of 0.56,[13] which was determined with a non-alcohol-producing yeast in a chemostat culture using a synthetic medium.

Simulation of Glucose-Stat Fed-Batch Culture

It has been widely accepted that a glucose-fed batch culture is more suitable than a batch culture for producing yeast cell mass, as the unfavorable effect of a high concentration of glucose-stimulating fermentative metabolism can be avoided by feeding glucose at a proper velocity. The feed rate of glucose or molasses is fixed empirically in the industrial manufacturing of bakers' yeast. The glucose feed rate as well as some chemical changes during a fed-batch yeast culture can be calculated by using the kinetic constants of yeast growth accumulated so far in this work. An example of simulation was attempted with a case where the glucose concentration in the culture is maintained at a steady-state value by feeding concentrated glucose solution until a certain amount of glucose was added to the fermentor. This type of fermentation control has not been performed by on-line methods, since the direct measurement and control of such low glucose concentrations in the culture is not yet possible.

The basic equations expressing the variations in concentrations of yeast cell mass (X), glucose (S), ethanol (A), and invertase (E) in a fed-batch culture are written as follows, according to the growth model developed previously with a slight modification for applying to a fed-batch culture and neglecting a time lag for growth:

$$\frac{dX}{dt} = G_S + G_A \tag{12}$$

$$-\frac{dS}{dt} = \frac{G_S}{Y_{X/S}(S)} \cdot \delta \tag{13}$$

$$S = \bar{S} \cdot (1 - \delta) + S \cdot \delta \tag{14}$$

$$\frac{dA}{dt} = -\frac{1}{Y_{X/A}^{resp}} \cdot G_A + \frac{Y_{A/S}^{ferm} R(S)}{Y_{X/S}(S)} \cdot G_S \tag{15}$$

$$\frac{dE}{dt} = \eta_S \cdot G_S + \eta_A \cdot G_A \tag{16}$$

$$\frac{dZ}{dt} = \frac{1}{Y_{X/S}(S)} \cdot G_S \cdot (1 - \delta) \tag{17}$$

where

$$Y_{X/S}(S) = [1 - R(S)] \cdot Y_{X/S}^{\text{resp}} + R(S) \cdot Y_{X/S}^{\text{ferm}} \tag{18}$$

and δ is a step function indicating that

$$\delta = 0 \text{ if } Z < Z_F \qquad \delta = 1 \text{ if } Z = Z_F \tag{19}$$

Z is the amount of glucose fed to a fermentor until time t divided by the fermentor liquid volume. Z_F is the total amount of glucose to be fed to the fermentor per liter of fermentor liquid.

These equations were solved with a computer (HITACHI 8700/8800, TODAI Center) for a case where 1 g glucose/liter fermentor liquid (Z_F = 1.0 g/liter) is fed to a fermentor to maintain a steady-state concentration of glucose: S = 0.1, 0.03, 0.02, and 0.015 g/liter. After glucose feeding is complete, the cultivation is continued as a batch culture until growth ceases due to the depletion of carbon source (residual glucose and produced ethanol). The glucose concentration at start-up is set at a steady-state value instantaneously by feeding an appropriate quantity of glucose from a medium reservoir. The volume change of fermentor liquid is considered as negligible during the culture, by feeding a very concentrated glucose medium. The initial conditions of the fed-batch culture are taken as X_0 = 0.012 g cells/liter, A_0 = 0.016 g ethanol/liter and $(E/X)_0$ = 5.8 kU/g cells in accord with the experimental condition for a fed-batch culture shown later. The kinetic parameters of K_S, K_A, $Q(S)$, and $R(S)$ determined before in a chemostat culture were used in the calculation. The other kinetic and stoichiometric constants used were those established in a batch culture (see Table II).

Figure 8 shows the results of the calculations. Figure 8(a) illustrates the growth curves in a batch culture and four runs of glucostat fed-batch cultures. In the batch culture, yeast grows up to 0.225 g/liter during 7.8 hr of incubation when glucose is depleted from the culture. The time of glucose depletion is shown by an arrow. Continued growth on consumption of ethanol accumulated so far in the culture (shown by a line after the arrow) results in a final cell yield of 0.410 g/liter at 14.4 hr. In a fed-batch culture, glucose is supplied

to the fermentor at a glucose feed rate controlled to maintain a constant concentration of residual glucose in the culture. Numbers 1-4 indicated in Figure 8 are concerned with the steady-state glucose concentration of 0.1, 0.03, 0.02, and 0.015 g/liter, respectively. Although a lower growth rate is observed with a lower steady-state glucose concentration, the cell mass concentration at the time of glucose depletion increases with a decrease in the glucose concentration: 0.250 (9.6 hr), 0.405 (14.7 hr), 0.480 (18.0 hr), and 0.505 (21.0 hr), respectively, in order with the decrease in the steady-state glucose concentration. However, the final cell yield increases but only slightly: 0.425 (15.0 hr), 0.480 (16.8 hr), 0.495 (19.0 hr), and 0.510 (21.6 hr). For maintaining each steady-state concentration of glucose during the fed-batch cultures, the mass feed rate of glucose per liter of fermentor liquid (Z) must be controlled as shown by the lines in Figure 8(b). Except for the later growth period, the glucose feed rate increases nearly exponentially with time. The first-order rate constant of the feeding corresponds to the specific growth rate of cells at each glucose concentration.

As long as the final cell yield is concerned, a fed-batch culture is not surprisingly advantageous in comparison to a batch culture. It is in a sense rather disadvantageous as the yeast growth rate is decreased remarkably. However, another growth yield, defined at the instant of glucose exhaustion from the culture, is much more improved in a fed-batch culture than in a batch culture. This is of course due to a reduction of ethanol formation by maintaining diluted glucose concentrations in the culture to decrease the Crabtree effect. As shown in Figure 8(c), the ethanol concentration in the culture can be regulated to as low as 0.015 g/liter by maintaining the glucose concentration in the culture below 0.015 g/liter, otherwise the ethanol concentration increases to 0.035–0.245 g/liter depending on the steady-state glucose concentration. The maximum coefficient of the improvement in the growth yield is $(0.505 - 0.012)/(0.225 - 0.012) = 2.3$ when glucose concentration is maintained at 0.015 g/liter. This definition of growth yield is important if one needs to harvest yeast cells with a high invertase activity. As illustrated in Figure 8(d), yeast cells have much more invertase content when grown in a glucose-containing medium (shown by the lines preceding the arrows) than in an ethanol medium (the lines after arrows). If one wants to obtain invertase-rich yeast in a batch culture, one must harvest cells at the end of the first growth phase even though the growth yield is rather low (55% of the final cell yield). One can elevate the harvest yield of invertase-rich yeast

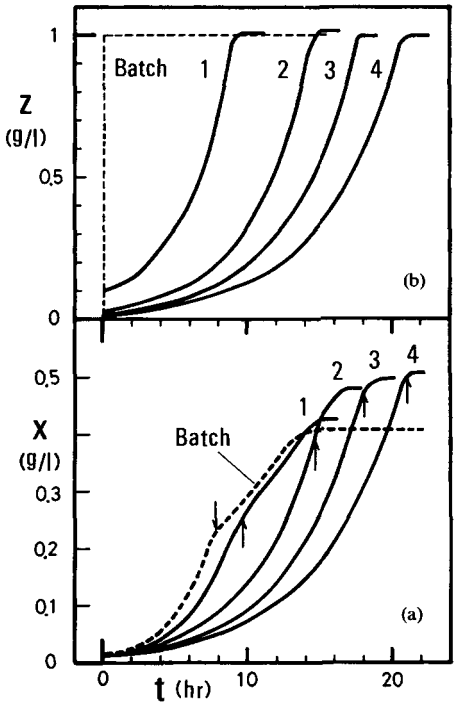

Fig. 8. Simulation of chemical changes in fed-batch culture of yeast. Glucose concentration in culture is maintained at 0.1 (indicated by number 1), 0.03 (number 2), 0.02 (number 3), and 0.015 (number 4) g/liter during the period of glucose feed. (a) Growth curves. Arrows indicate the time of depletion of glucose from the culture. Yeast grows on ethanol after the arrows. Growth curve of a batch culture is also shown as a dotted line for reference. (b) Mode of glucose feeding to maintain steady-state concentration of glucose in the culture. (c) Variation of ethanol concentration in the culture. (d) Variation of specific invertase activity in yeast cells.

about 2.3-fold in a batch culture by the technique of fed-batch culture, where the glucose concentration must be controlled below 0.015 g/liter. Therefore, one of the merits of fed-batch culture would be improving the quality of the cells in addition to increasing the growth yield, although the growth yield itself can be increased more by extending the cultivation time in a batch culture, if one does not care for the properties of yeast grown on the product ethanol remaining in the culture.

Glucose Fed-Batch Culture

Figure 9 shows an experimental run of fed-batch yeast culture with stepwise feeding of fresh media containing different glucose

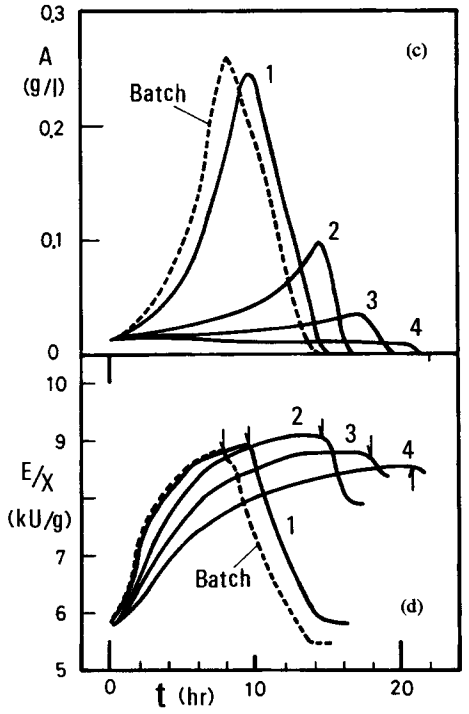

Fig. 8. (*Continued from previous page.*)

concentration at a constant liquid flow rate of 6.5 ml/hr into 1.5 liter culture medium, which initially contained 10% (v/v) inoculum culture and no glucose. The glucose concentration in the feed medium was doubled in regular intervals from 0.59 g/liter at 0 hr to 75.7 g/liter at 14 hr. Figure 9(a) shows the quantity of glucose added to the fermentor divided by the liquid volume [$Z = \int (S_R F/V)\, dt$] as a dotted line, together with the change of glucose concentration in the culture as open circles. The curve for glucose consumption in a batch culture is also shown as closed circles for comparison. The variations in cell mass concentration and ethanol concentration in the culture as well as the specific invertase activity of yeast are shown in Figures 9(b)–9(d), respectively. As the glucose feeding mode was unfortunately not ideal in this experimental run for reproducing a steady-state glucose concentration in the culture, which would be realized if exponential feed of glucose medium was performed, a considerable amount of ethanol was produced at the later period of cultivation (14–18 hr), thus resulting in a slight decrease

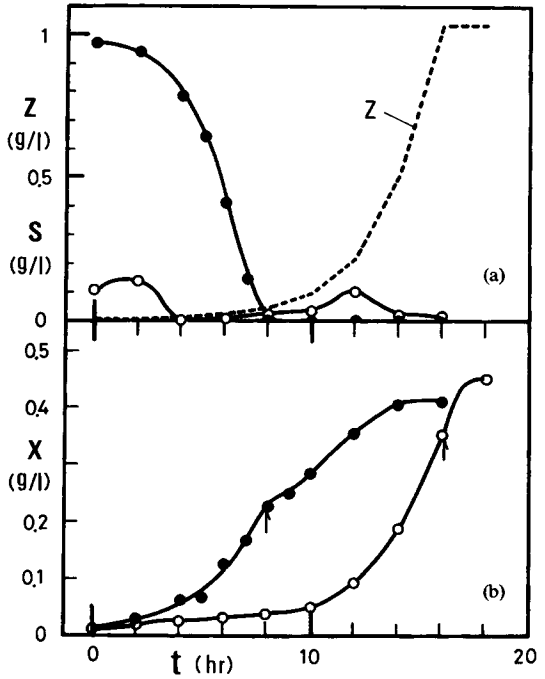

Fig. 9. Experimental result of glucose-fed batch culture of yeast (○). Data obtained in a batch culture (●) are shown for comparison. (a) (— — —) Feed rate of glucose changed doubly in time intervals of 2 hr. Variations in glucose concentration are represented by (○) (fed-batch) and (●) (batch). (b) Change in cell mass concentration. Arrows indicate the time of glucose exhaustion from the culture. (c) Change in ethanol concentration. (d) Change in specific activity of invertase in yeast cells.

of specific invertase activity in the harvested cells. The unexpected result of high ethanol concentration in spite of the fact that the glucose concentration was maintained as dilute as 0.03 g/liter at the time period of 14–16 hr, may be ascribed to the instantaneous and sawtooth-like change of glucose concentration in the culture during a short period of several seconds between dropwise addition of very concentrated glucose medium by a peristaltic pump into the fermentor liquid. Except for the above, the tendency of the experiment is quite similar to the prediction of a fed-batch culture as shown in Figure 8.

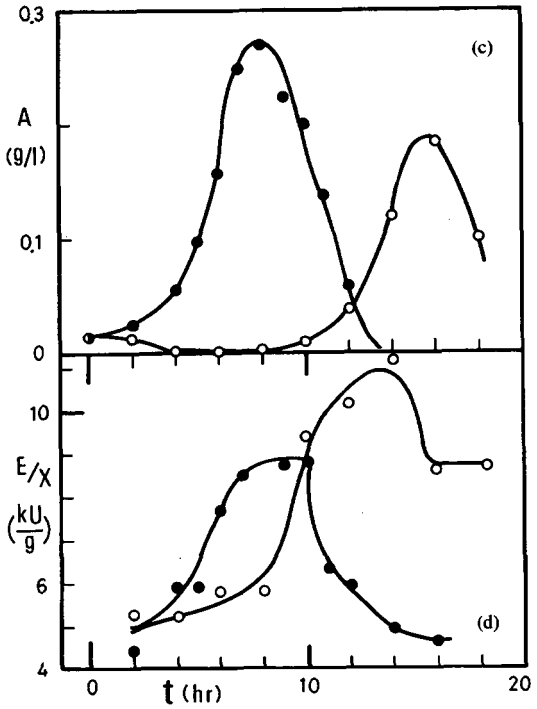

Fig. 9. (*Continued from previous page.*)

Nomenclature

A	ethanol concentration in culture filtrate (g/liter)
A_R	ethanol concentration in medium reservoir (g/liter)
D	dilution rate (hr^{-1})
E	invertase activity in culture (kU/liter)
E/X	specific invertase activity of yeast (kU/g cells)
F	feed rate of fresh medium (ml/hr)
G_S	growth rate on glucose consumption (g cells/hr/liter [see eq. (1')]
G_A	growth rate on ethanol consumption (g cells/hr/liter [see eq. (1')]
K_A	ethanol saturation constant (g/liter)
K_S	glucose saturation constant (g/liter)
K_La	volumetric oxygen transfer coefficient or ethanol desorption (min^{-1} or hr^{-1})
$Q(S)$	coefficient for repression of ethanol utilization in yeast by glucose (dimensionless)
$R(S)$	ratio of fermented glucose in total glucose consumed by yeast (dimensionless)

S	glucose concentration in culture filtrate (g/liter)
S_R	glucose concentration in medium reservoir (g/liter)
t	time (hr)
V	volume of fermentor liquid (liter)
X	concentration of dried yeast biomass in culture (g/liter)
$Y_{X/S}(s)$	yield of yeast biomass per glucose utilized (g cells/g glucose)
$Y_{X/A}^{resp}$	yield of yeast biomass per ethanol consumed (g cells/g ethanol)
$Y_{X/S}^{resp}$	yield of yeast biomass per respired glucose (g cells/g glucose respired)
$Y_{X/S}^{ferm}$	yield of yeast biomass per fermented glucose (g cells/g glucose fermented)
$Y_{A/S}^{ferm}$	yield of ethanol per fermented glucose (g ethanol/g glucose fermented)
Z	amount of glucose fed per unit volume of fermentor liquid (g/liter)

Greek

δ	step function defined in eq. (19) (dimensionless)
η	ratio of invertase formation rate to growth rate (kU/g cells)
μ_A^{max}	maximum specific rate of growth on ethanol (hr^{-1})
μ_S^{max}	maximum specific rate of growth on glucose (hr^{-1})

Super- and Subscripts

$\overline{\quad}$	average value
-	value at steady state
0	initial value
max	maximum value
F	final value
A	concerned with ethanol
S	concerned with glucose
X	concerned with cell
resp	concerned with respiration
ferm	concerned with fermentation

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