APPLICATION OF FUZZY CONTROL SYSTEM TO COENZYME Q_{10} FERMENTATION

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A fuzzy control system for a fed-batch culture was constructed and applied to coenzyme Q_{10} fermentation. For inputs, cell concentration ([OD] and [OD2]), specific growth rate ([SGR]) and fermentation time ([BTIM]) were selected. A base value of aeration rate ([AIRB]) and an adjustment term for [AIRB] ([DAIR]) were defined as outputs. In this system, cell concentration was measured on-line by a turbidimeter and specific growth rate was then calculated. After cell concentration, specific growth rate and fermentation time were transferred into four inputs using membership functions, and these were further transferred into two outputs by 70 fuzzy rules. After defuzzification of [AIRB] and [DAIR], a set value of the aeration rate was determined as the sum of defuzzied [AIRB] and [DAIR]. In this fuzzy control system, the aeration rate was set at two-hour intervals according to its state and the productivity was higher and had less deviation than model fermentation (predetermined stepwise shift up each 20 hours in the aeration condition). Such result was also obtained even in the case of low initial cell concentration. These data indicated the usability and stability of this fuzzy control system.

Introduction

The modeling of a fermentation process is difficult because of the many biosynthetic reactions contained in it. A computer-controlled fermentation of glutamic acid using a dynamic programming method has been reported[2], but in many cases there are numerous difficulties in constructing a mathematical model of a computer-coutrolled fermentation process.

Recently, fuzzy reasoning as a control theory has

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been applied to many kinds of processes. In these processes, relations between state variables and control variables cannot be determined by simple functions, and operations in these processes usually depend on expert experience and imagination. In that sense, fermentation seems to be a most appropriate kind of process for application of fuzzy control theory, and several examples have been reported[4]. In these applications, however, fuzzy reasoning was used mainly to respond to an unusual state in the process. We have tried to apply fuzzy reasoning to the determination of operation conditions using on-line data and to control the fermentations so as to maintain the desirable condition.

Here the fuzzy control system was applied to coenzyme Q_{10} fermentation. This process was carried out under the limitation of oxygen supply, and was controlled by the oxygen supply condition when the substrates were fully supplied[3]. Oxidation-reduction potential (ORP) values reached a constant level of about -100 (mv), which indicated that the dissolved oxygen concentration in the fermentation broth was almost equal to zero (**Fig. 1**), and the productivity (defined as the concentration of coenzyme Q_{10} per unit volume of the fermentation broth) and the cell concentration were regulated mainly by the rate of oxygen supply (**Fig. 2**). Hence the oxygen supply condition was selected as a control variable in the fuzzy controller. The cell concentration was defined as a state variable, because coenzyme Q_{10} is an intracellular product and no method of direct on-line measurement has been developed, whereas on-line determination of cell concentration is possible by measuring suspended solid (SS) concentration using a turbidimeter. A fuzzy controller was constructed to achieve a more stable fermentation process. Fermentation experiments were carried out using this fuzzy controller, and its stability was investigated.

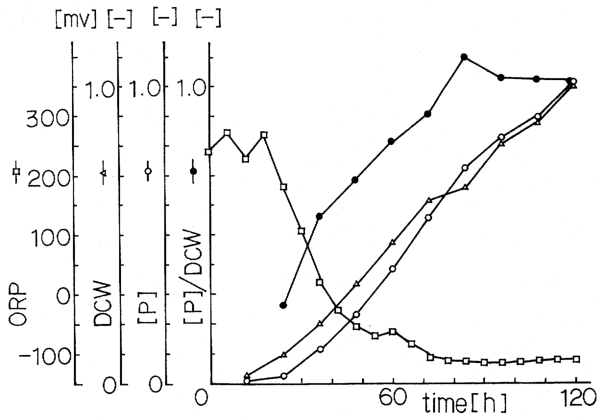

Fig. 1. Time course of oxidation-reduction potential (ORP), dry cell weight (DCW), productivity ([P]) and content of coenzyme Q_{10} per unit cell ([P]/DCW) in a model fermentation

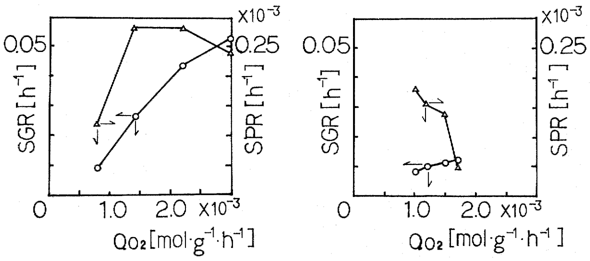

Fig. 2. Relationship between a specific growth rate (SGR) or specific production rate (SPR) and specific oxygen uptake rate (Q_{O2})
(a) in a logarithmic growth phase (b) in a transitoin phase

1. Materials and Methods

1.1 Microorganism and cultivation

A strain derived from *Rhodobacter* sp. was used. Fermentation was carried out in a semisynthetic medium in which glucose aqueous solution (68%) as the main carbon source was fed continuously to the broth, because cell growth and coenzyme Q_{10} production were depressed under depletion of the substrate, and the glucose concentration in the broth was maintained at around 2 to 3%. The pH was maintained at 6.4 by aqueous ammonia and the temperature was 32°C. The internal pressure was maintained at $2.94*10^4$ N/m^2 (gauge pressure).

1.2 Fermenters

30 l-jar fermenters with turbine impellers were used and the working volume was 20 l.

1.3 Oxygen supply

The rate of oxygen dissolution was expressed by the balance of oxygen supply condition and oxygen uptake rate, R_{ab}, as shown in Eq. (1).

$$dC/dt = k_La(C^* - C) - R_{ab} \tag{1}$$

In Eq. (1), C is the oxygen concentration in the broth and C* is the saturated value of C. In coenzyme Q_{10} fermentation, C and dC/dt are nearly equal to zero under the limitation of the oxygen supply condition. As a result, the rate of oxygen supply to the cell was determined as the oxygen absorption rate, k_LaC^*, which was measured by the sodium sulfite oxidation method[1]. The following equation was obtained as an exponential function of aeration rate (Q) and agitation rate (N).

$$k_LaC^* = 2.27*10^{-8}*N^{2.45}*Q^{0.38} \tag{2}$$

Here, C* was assumed to be equal to the saturated oxygen concentration in the water at 32°C. Equation (2) indicates that k_LaC^* can be widely varied by the agitation rate and fine adjustment is possible by control of the aeration rate. Hence the aeration rate was selected as the main control variable in the fuzzy control system, and if the aeration rate exceeded the upper limit, the agitation rate was shifted from 200 min^{-1}, to 240 min^{-1}, followed by recalculation of the aeration rate using Eq. (2).

R_{ab} was calculated by multiplying the aeration rate by the oxygen concentration in the exhaust gas, which was measured by a gas analyzer (Fuji Electric Co., Ltd.).

1.4 Computer-controlled fermentation system

The computer-controlled fermentation system consisted of 1) four fermenters, 2) a computer for process control (YEWPACK, Yokogawa Hokushin Electric Co., Ltd.), 3) two interface units between the process computer and the fermenters (UFCH, Yokogawa Hokushin Electric Co., Ltd.), 4) a computer for calculation of fuzzy reasoning (N-5200, NEC Corporation), and 5) an interface unit between the two computers (UGWU, Yokogawa Hokushin Electric Co., Ltd.) as shown in **Fig. 3**. In this system, the process values were maintained at the set values by YEWPACK through UFCH and signal conditioners. Predetermined set values of temperature, pH and internal pressure were kept constant throughout the whole process, but aeration and agitation rates were changed from the initial value during the fermentation. Process values were sent to N-5200 through UGWU, and the fuzzy reasoning to determine the aeration rate using SS concentration measured by a turbidimeter (SSB-50, DKK Co., Ltd) was carried out on N-5200 at two-hour intervals. Here, measurement by turbidimeter was carried out every two minutes, and the moving average of five data was used as the SS concentration. Results of fuzzy reasoning (set value of aeration rate) were sent back to YEWPACK through UGWU.

1.5 Analysis

Cell concentration (off-line data) was measured as dry cell weight (DCW) using 5 ml of fermentation broth. Coenzyme Q_{10} concentration in the broth was measured by HPLC after extracting the coenzyme Q_{10} from the cells by 2-butanol (pH 2.0). These data were expressed as relative values divided by the mean values in the model pattern.

2. Results

2.1 Outline of fuzzy control system

An outline of the fuzzy control system applied to coenzyme Q_{10} fermentation is shown in **Fig. 4**. Cell concentration was measured on-line by a turbidimeter using a calibration curve, and the specific growth rate (SGR) was calculated by Eq. (3), where X_t and X_{X-2} are the cell concentration at t[h] and t−2[h] respectively.

$$SGR = (X_t - X_{t-2})/(2*(X_t + X_{t-2})/2) = (X_t - X_{t-2})/(X_t + X_{t-2}) \quad (3)$$

Cell concentration, specific growth rate and fermentation time were tansferred into corresponding fuzzy variables ([OD], [OD2], [SGR] and [BTIM]) by membership functions. Those inputs were further transferred into outputs ([AIRB] and [DAIR]) using fuzzy rules. After defuzzification of these fuzzy variables, the set value of the aeration rate was calculated by the sum of defuzzied [AIRB] and [DAIR].

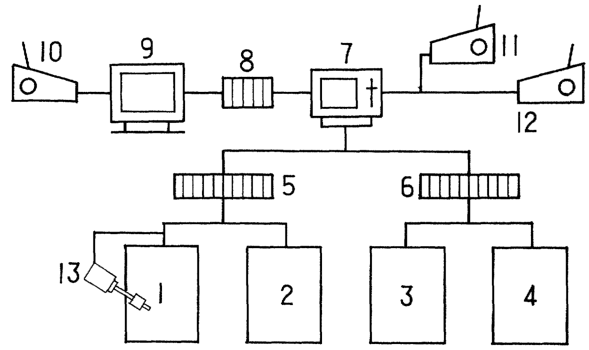

Fig. 3. Schematic diagram of computer-controlled fermenters: 1—4, fermenters; 5—6, UFCH; 7, YEWPACK 8, UGWU; 9, N-5200; 10—12, printers; 13, turbidimeter

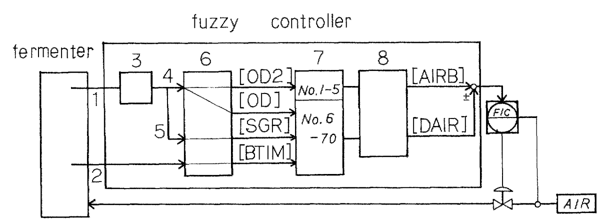

Fig. 4. Constitution of fuzzy controller: 1, SS concentration data; 2, fermentation time; 3, calibration curve; 4, measured cell concentration; 5, calculated specific growth rate; 6, membership functions of inputs and outputs; 7, 70 fuzzy rules 8, defuzzification block

2.2 Construction of fuzzy controller

The initial fuzzy controller was defined temporarily on the besis of experience and knowledge of the process. The time course of aeration condition where the highest productivity had been obtained in the past was selected as a model pattern. Using the initial fuzzy controller, the aeration rate (the sum of defuzzied [AIRB] and [DAIR]) was calculated by the cell concentration of the model pattern. Tuning of the initial fuzzy controller was carried out by modifying membership functions of inputs and outputs, and as a result the calculated aeration rate was almost the same as the aeration condition in the model. After tuning, fermentations using this fuzzy controller were carried out, and membership functions and fuzzy rules were further modified based on the fifference between the model and fuzzy control fermentation, and on the knowledge acquired. Their final definitions are shown in **Fig. 5** and **Table 1.**

State variables in the fuzzy controller were defined by the cell concentration and the specific growth rate

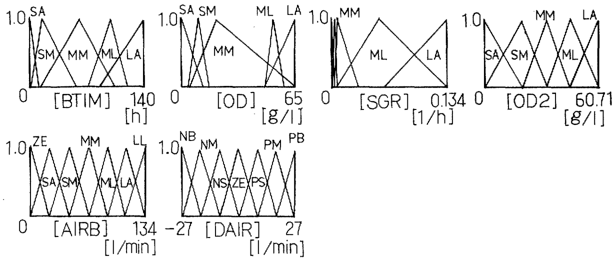

Fig. 5. Membership functions of inputs and outputs: ZE, "zero"; SA, "small", SM, "small medium"; MM, "medium"; ML, "medium large"; LA, "large"; LL, "very large"; NB, "negative big"; NM, "negative medium"; NS, "negative small"; PS, "positive small"; PM, "positive medium"; PB, "positive big"

Table 1. Examples of fuzzy rules

rule No.	inputs				outputs	
	[BTIM]	[OD]	[SGR]	[OD2]	[AIRB]	[DAIR]
1				SA	ZE	
2				SM	SA	
3				MM	SM	
4				ML	MM	
5				LA	ML	
31	MM	SA	SA			PB
32	MM	SM	SM			PB
33	MM	SM	MM			PM
34	MM	SM	ML			PM
35	MM	SM	LA			PS

at the appropriate time. Membership functions of both inputs and outputs are shown in Fig. 5. Values on the abscissa axis are the values of the variables, and the grade, representing the extent to which the value is contained in the corresponding fuzzy set (SA, SM. etc.), is shown on the ordinate. In this figure, [BTIM] and [SGR] are the fuzzy variables of fermentation time and specific growth rate, respectively. Both [OD] and [OD2] are the fuzzy variables of the cell concentration, but are adopted by different rules and used for the calculation of [DAIR] and [AIRB] respectively. [OD2] is needed for determination of the fundamental oxygen supply condition corresponding to the amount of cell mass, whereas [OD] is used as an index of the fermentation phase (e.g. lag phase, log phase, transition phase). Linguistic fermentation states and operation are transferred into fuzzy sets by these membership functions.

The relationship, known by operators and us, between the inputs ([OD2], [OD], [SGR] and [BTIM] and the outputs ([AIRB] and [DAIR]) are defined by fuzzy rules. Several examples of these rules are shown in Table 1, in which the variables have the same meaning as in Fig. 5. [AIRB] is the base value determined approximately proportional to [OD2] by five reles (No. 1 to No. 5), and [DAIR] is the adjustment term for the base value determined from

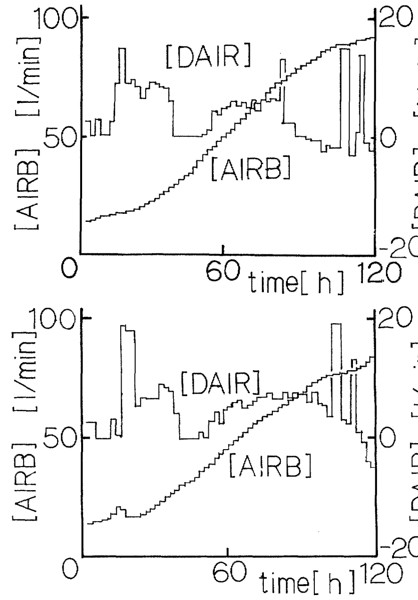

Fig. 6. Time course of calculated [AIRB] and [DAIR]

the state of fermentation ([OD], [SGR] and [BTIM]) by 65 rules (No. 6 to No. 70). The grade of output's fuzzy set is determined as the minimum value among the grades of inputs fuzzy sets by each selected rule. If more than one value of the grade is obtained for an output's fuzzy set, the grade is determined as the maximum value among them. Using these fuzzy rules, corresponding fuzzy sets and grades are determined for [AIRB] and [DAIR]. Defuzzification of each variable is then carried out, using a simplified center-of-gravity method. The set value of the aeration rate is defined as the sum of defuzzied [AIRB] and [DAIR].

2.3 Fermentation by fuzzy control system

The fuzzy control system thus constructed was applied to coenzyme Q_{10} fermentation. Two examples of calculated outputs ([AIRB] and [DAIR]) are shown in **Fig. 6.** Characteristic features of the time course of [DAIR] were the existence of two posivive peaks (10—40 h and 60—90 h) and negative values after 100 h. Positive peaks reflected the rules which attempted to maintain a higher cell growth rate by increasing the aeration rate. Negative values were a result of adopting rules that attempted to increase the specific production rate (production rate per unit cell) by limiting the aeration rate.

Cell conentration in the fuzzy control system was kept higher than that in the model pattern (scheduled stepwise shift up each 20 h in the aeration condition), and so was final productivity corresponding to the increment of final DCW (**Figs. 7 and 8**). In four experiments, the final DCW and productivity in the fuzzy control system were almost equal to or higher than those in the model fermentation (**Table 2**). In the case of the fuzzy control system, limitation of R_{ab} by

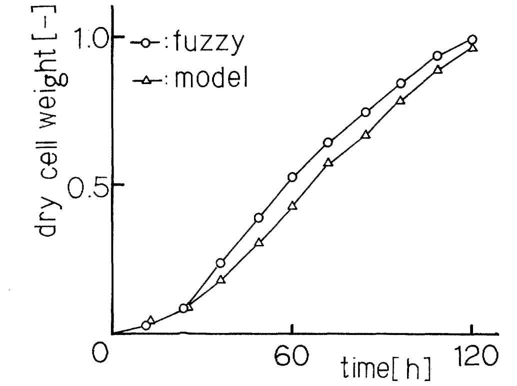

Fig. 7. Time course of dry cell weight (DCW)

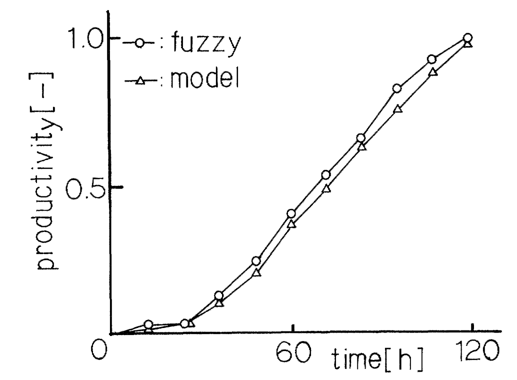

Fig. 8. Time course of productivity

Table 2. Results of fermentation by fuzzy control system and model pattern

Batch. No.	DCW		productivity	
	fuzzy	model	fuzzy	model
FZ-8	1.058	0.986	1.009	0.882
FZ-13	1.113	1.034	1.168	1.120
FZ-15	1.000	0.980	0.991	0.981
FZ-16	0.983	1.000	1.013	1.015
mean value	1.039	1.000	1.045	1.000
deviation	0.051	0.021	0.071	0.085

k_LaC^* was less than that in the model fermentation, especially in the early period of the fermentation (**Fig. 9**).

2.4 Fermentation with low initial cell concentration

The stability of the fuzzy control system was examined from another point of view. A time delay in the fermentation was induced artificially by decreasing the initial cell concentration (i.e. the amount of broth transferred from a seed tank to the jar fermenters) to one-fourth that of the model fermentation. The resulting time delay was estimated as about 10—15 h from the doubling time calculated from the initial specific growth rate. In the fermentation using the fuzzy control system, rapid recovery from the initial delay, especially for cell

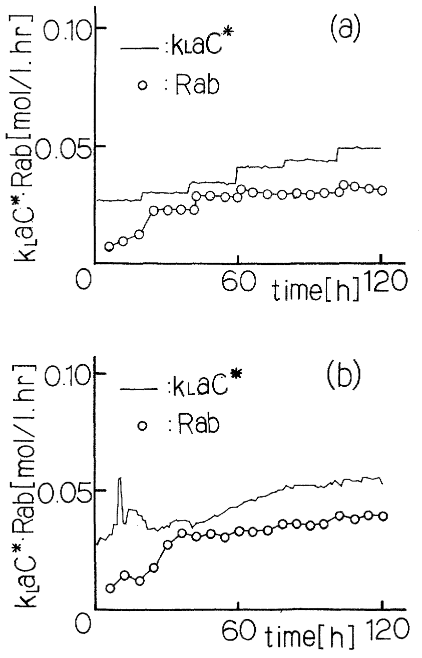

Fig. 9. Time course of k_LaC^* and Rab: (a), model pattern; (b), fuzzy control system

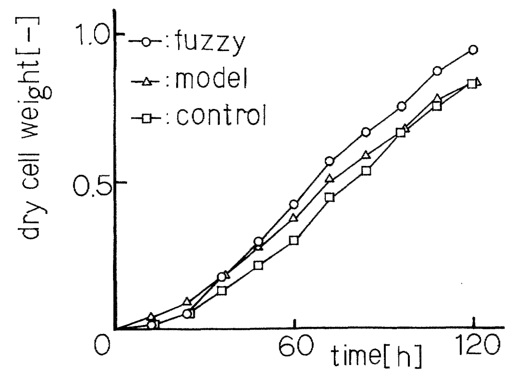

Fig. 10. Time course of dry cell weight (with low initial cell concentration)

concentration, was observed, while in the "control" fermentation, which was set at the same conditions as the model fermentation pattern with one-fourth the initial cell concentration, it took more time to catch up with the time course of the model fermentation (**Figs. 10 and 11**).

3. Discussion

In our study, possibilities of the application of fuzzy reasoning to the on-line determination of fermentation conditions in a computer-controlled coenzyme Q_{10} fermentation system were examined calculated aeration rate by the fuzzy control system consisted of a base value, [AIRB], and an adjustment term, [DAIR]. Modification of fuzzy rules was thought to be easier by separating [DAIR] from [AIRB], because [DAIR] represents experience and knowledge of the process. [AIRB] was needed to determine the

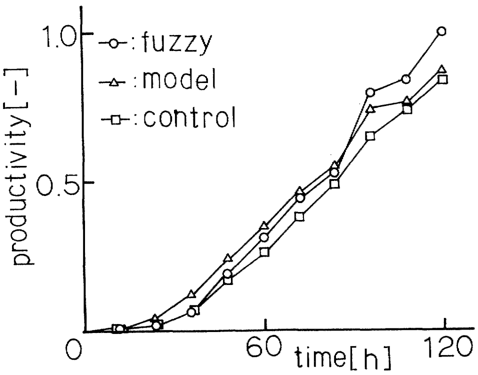

Fig. 11. Time course of productivity (with low initial cell concentration)

fundamental valume of aeration rate corresponding to the amount of cells and [DAIR] was useful for responding to the variation in growth activity. It was thus possible by calculating [AIRB] and [DAIR] separately to determine the aeration rate according to the aeration rate according to the variation of the fermentation state, e.g. low cell concentration at the average gwowth rate or low growth activity at the average cell concentration.

In fuzzy control fermentation, rules to maintain a high growth rate were mainly adopted at first, and final productivity became higher than in the model fermentation due to higher cell concentration. After about 100 h, positive values of [DAIR] sometimes appeared (Fig. 6), because SS concentration did not vary greatly and specific growth rate was calculated to be almost equal to zero. In such a case, rules to increase growth rate by setting a higher aeration rate were adopted, and [DAIR] was defined as PB (positive big) or PM (positive medium). Growth rate at an adequate level was recovered, and then the value of [DAIR] became negative. As a result, high productivity was maintained even in the late period of fermentation.

In the membership function of [DAIR] (Fig. 5), the maximum value was changed from the initial value of the abscissa (9 *l*/min) to 27 *l*/min to intensify the effect of [DAIR]. This may be one reason why the pattern of oxygen supply became a little different from that in the model fermentation. Coenzyme Q_{10} fermentation could be carried out more stably by applying this fuzzy control system as shown in Table 2, resulting in a slight increase in final productivity.

We have discussed here the coenzyme Q_{10} fermentation, where no method for on-line measurement of the intracellular product has been developed. We therefore used the cell concentration as a state variable instead of the coenzyme Q_{10} concentration (objective function), through the relationship between the objective function and the outputs was not so clear. In that sense, fuzzy reasoning is throught to be easily applicable to a process where on-line measurement of products is not possible and the relationships between productivity and other measurable variables are not completely explained.

Nomenclature

[AIRB]	= base value of aeration rate calculated by fuzzy controller	[*l*/min]
[BTIM]	= fermentation time (input in fuzzy controller)	[h]
C	= dissoved oxygen concentration in broth	[kmol/m^3]
C*	= saturated dissolved oxygen concentration in water at 32°C	[kmol/m^3]
[DAIR]	= adjustment term of aeration rate calculated by fuzzy controller	[*l*/min]
dC/dt	= oxygen dissolution rate	[kmol/m^3/h]
DCW	= dry cell weight	[—]
k_La	= coefficient of oxygen transfer rate	[h^{-1}]
N	= agitation rate	[min^{-1}]
[OD]	= cell concentration to determine [DAIR] (input in fuzzy controller)	[g/*l*]
[OD2]	= cell concentration to determine [AIRB] (input in fuzzy controller)	[g/*l*]
ORP	= oxidation-reduction potential	[mV]
[P]	= productivity	[—]
Q	= aeration rate	[*l*/min]
Q_{O2}	= specific respiration rate	[kmol/g/h]
R_{ab}	= oxygen uptake rate ($=Q_{O2}*X_t$)	[kmol/m^3/h]
SGR	= specific growth rate	[h^{-1}]
[SGR]	= specific growth rate (input in fuzzy controller)	[h^{-1}]
SPR	= specific production rate	[h^{-1}]
SS	= suspended solid	[—]
X_t	= cell concentration at t[h]	[—]
X_{t-2}	= cell concentration at t−2[h]	[—]

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