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Anaerobic-Light Growth Kinetics of *Rhodopseudomonas sphaeroides S*

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ABSTRACT

The inhibitory effects of propionate and acetate, and the enhancing ability of light on the growth rate of *Rhodopseudomonas sphaeroides S* were investigated in a modified turbidostat culture apparatus at different substrate concentrations and the light intensities. The experiments were carried out at 30 °C under anaerobic-light condition. Compared to acetic acid, propionic acid strongly inhibited the anaerobic-light growth of *Rhodopseudomonas sphaeroides S*. The μ_m observed about 0.4 h^{-1} from both acids, was considerably high at very low cell concentration of 12 mg/l and 3000 lux. In simulating the inhibition data, the modified equation of Haldane, $\mu = \frac{\mu_m}{1 + \frac{K_S}{C_S} + (\frac{C_S}{K_i})^n}$ and the generalized non-competitive inhibition model, $\mu = \frac{\mu_m}{(1 + \frac{K_S}{C_S})(1 + (\frac{C_S}{K_i})^n)}$ were the most suitable among the five models employed. From these equations, the inhibition constant, K_i and n increased with the increase in light intensity. This implied that the inhibitory effects of high concentration of propionic and acetic acids could be minimized when sufficient light energy was supplied.

Key words: growth kinetics, *Rhodopseudomonas sphaeroides S*, photosynthetic bacteria

INTRODUCTION

Several previous workers (Aiba *et al.*,1979; Nishizawa *et al.*, 1974; Sasaki *et al.*, 1978; Sasaki and Nagai, 1979) carried out experiments dealing with *Rhodopseudomonas sphaeroides S*, a specific species of non-sulfur purple bacteria. This strain is capable of growth either under aerobic-dark or anaerobic-light conditions (D' Adamo *et al.*, 1984; Edwards, 1970). This is known to metabolize fatty acids of low molecular weight such as acetate and propionate and can accumulate vitamin B_{12} intracellularly to an extent of 23 g/g cell (Morikawa *et al.*, 1971) or 34 g/g cell or 67 g/g cell (Sasaki *et al.*, 1978) under aerobic-dark condition in n-paraffin, propionate, or propionate-acetate media, respectively. Also, an attempt to apply this bacteria in waste treatment from food industry and for the production of biomass as an animal feed with high nutritive value were reported (Sasaki *et al.*, 1978).

Studies on the utilization of acetate and/or propionate revealed that this organism grows favorably in medium containing either acetate or a mixture of acetate and propionate, but not in the medium containing propionate as a sole carbon source. The growth yield in anaerobic-light cultivation of *Rhp. sphaeroides S* was two times as large as in aerobic-dark condition, using glucose and lower fatty acids as single or mixture of carbon sources, but the specific growth rate was reversed (Sasaki *et al.*, 1978). Other previous studies have focused on the metabolic and/or enzymatic activities of this bacterium, growth yields (Nishizawa *et al.*, 1974) and vitamin B_{12} synthesis (Sasaki *et al.*, 1978; Sasaki and Nagai, 1979).

Most of the previous workers have not yet done any studies on the growth inhibition of

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Rhodopseudomonas sphaeroides S at higher concentration of these fatty acids. In the present work, further studies were conducted to elucidate the substrate inhibition of propionic or acetic acids to the growth rate using turbidostat culture technique under anaerobic-light condition. The suitable cell mass, propionate, acetate and yeast extract concentrations for growth were determined. The effects of light intensity in decreasing the inhibitory effects at high substrate concentrations were studied. Finally, the inhibition data were fitted to the proposed substrate inhibition models for the determination of kinetic constants.

THEORY

High substrate concentration inhibits growth and distorts the metabolism of microorganism, especially its growth on inhibitory carbon sources such as phenols, thiocyanates, nitrates, ammonia and volatile acids. The utilization of biological processes for treatment of certain industrial wastes containing those inhibitory substrates emphasizes the practical necessity for developing adequate mathematical models for design and operation of these processes. An attention is given in obtaining and analyzing experimental data for the purpose of determining the values of kinetic constants for use in the models.

Mathematical models of the growth of microorganism are generally based on the simple Monod model, representing the saturated phenomenon of growth rate and having 2 or 3 parameters. But a Monod type model can not present lag phase or the declining phase. The mathematical models under consideration herein, as proposed by previous workers, dealt with substrate inhibition kinetics and they depicted the relationship between specific growth rate (μ) and substrate concentration (C_S). These models are used in the present work.

In noninhibited growth, described by the Monod model, μ approaches an upper limit (μ_m) as C_S increases. On the other hand, substrate inhibition implies that, following an initial increase, μ must eventually decline as the substrate concentration increases.

Haldane (1965) derived a kinetic model which describes the case of competitive inhibition by the substrate itself. This model is given by Eq. (1) (Edwards, 1970).

$$V = \frac{V_m}{1 + \frac{K_1}{C_S} + \frac{(C_S)^{n-1}}{K_n}} \quad (1)$$

where V = velocity of reaction
C_s = substrate concentration
V_m, K_1, K_n = constants

This equation represents an enzyme forming an inactive enzyme-substrate complex involving two molecules of substrate per enzyme molecule at sufficiently high substrate concentration.

Yano and Koga (1973) have generalized the above case considered by Haldane (1965) by assuming the formation of multiple inactive enzyme-substrate complexes as follows:

$$(1)\ E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2'} E + P$$

$$(2)\ ES + S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_2 \text{ (inactive)}$$

$$(3)\ ES_2 + S \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} ES_3 \text{ (inactive)}$$

$$(n)\ ES_{n-1} + S \underset{k_{n-1}}{\overset{k_n}{\rightleftharpoons}} ES_n \text{ (inactive)}$$

The application of the conventional steady-state analysis and analogy of the substrate inhibition of microbial growth by this mechanism results in a model shown in Eq. (2) (D'Adamo *et al.*, 1984; Edwards, 1970; Sokol and Howell, 1981; Takamatsu *et al.*, 1981; Yang and Humphrey, 1975).

$$\mu = \frac{\mu_m}{1 + \frac{K_S}{C_S} + \sum_{j=1}^{n} (\frac{C_S}{K_j})^n} \quad (2)$$

where μ = specific growth rate
μ_m = maximum specific growth rate in the absence of inhibition

C_s = limiting substrate concentration
K_s = saturation constant; the lowest substrate concentration at which, in the absence of inhibition, the specific growth rate is half the maximum specific growth rate
K_j = kinetic parameter constant; j = 1, 2, 3, ..., n
n = positive integer; represents the order of inhibition by substrate itself

Yamane *et al.* (1976) proposed and adopted this empirical equation to study the stability of the exponential fed-batch culture when high concentration of substrate inhibits growth of microorganisms. Equation (2) can be simplified to equation (3) when K_1, K_2, ...K_{n-1} are of infinite values.

$$\mu = \frac{\mu_m}{1 + \frac{K_S}{C_S} + (\frac{C_S}{K_i})^n} \quad (3)$$

where K_i = inhibition constant; numerically equals to the highest substrate concentration at which the specific growth rate is equal to one-half the maximum specific growth rate in the absence of inhibition

For the study of the influence of high substrate concentrations on microbial kinetics, Edwards (1970) proposed the model shown in Eq. (4).

$$\mu = \frac{\mu_m}{1 + \frac{K_S}{C_S} + (\frac{C_S}{K_i})(1 + \frac{C_S}{K})} \quad (4)$$

Noncompetitive substrate inhibition (Edwards, 1970; Nanba *et al.*, 1983; Nanba *et al.*, 1984; Takamatsu *et al.*, 1981; Yano and Koga, 1973) is a result when the enzyme at another site which is sufficiently far away from the active center binding site such that there is no effect on the kinetics of formation and dissociation of the effective ES complex. This is an example of allosteric substrate inhibition where two ineffective enzyme-substrate complexes, SE and SES occur.

Eq. (5) has been evaluated by Edwards (1970) for its applicability to several substrate-inhibited microbial growth systems.

$$\mu = \frac{\mu_m C_S}{(K_S + C_S)(1 + \frac{C_S}{K_i})} \quad (5)$$

The general expression of this noncompetitive model (Nanba *et al.*, 1983; Takamatsu *et al.*, 1981; Yano and Koga, 1973) is given in Eq. (6).

$$\mu = \frac{\mu_m C_S}{(K_S + C_S)(1 + (\frac{C_S}{K_i})^n)} \quad (6)$$

In the situation when $K_S << C_S$ and substrate inhibition are considered, Eq. (6) is reduced to

$$\mu = \frac{\mu_m}{1 + (\frac{C_S}{K_i})^n} \quad (7)$$

This model is identical with that of Eq. (3) at high substrate concentration, when K_S is small and $\frac{K_S}{C_S}$ is negligible. Therefore, these two conceptually different models are experimentally indistinguishable.

The inhibition model, given by Eq. (7) has been used well to experimental data of Nanba *et al.* (1983; 1984), who studied the product inhibition of *Propionibacterium shermanii* (Nanba *et al.*, 1983) and substrate inhibition of *Acetobacter sp.* (Nanba *et al.*, 1984).

Edwards (1970) has proposed a similar expression which was derived from an empirical relationship of product inhibition developed by Aiba *et al.* (1979) for substrate inhibition. This is shown in Eq. (8).

$$\mu = \frac{\mu_m C_S \exp^{(-\frac{C_S}{K_i})}}{K_S + C_S} \quad (8)$$

Furthermore, Eq. (9) has also been proposed for substrate inhibition data by Edwards (1970) who combined the two mechanisms of diffusion-controlled substrate supply and protective diffusion-limitation of high and inhibitory substrate concentrations.

$$\mu = \mu_m (\exp^{(-\frac{C_S}{K_i})} - \exp^{(-\frac{C_S}{K_S})}) \quad (9)$$

These inhibition models given previously were used in this experiment and are summarized in Table 1. They were fitted to substrate inhibition data by minimizing the sum of squares from the difference between the observed data and the calculated values obtained using the models at each substrate concentration. The formula in Eq. (10) was used to estimate the goodness of fit.

$$SS = \sum_{i=1}^{n} (\text{Observed value} - \text{calculated value})^2 \quad (10)$$

where SS = sum of squares
n = number of data points

The nonlinear least squares regression technique was utilized to fit the data and to estimate the kinetic constants. These nonlinear least square fits were obtained and graphically drawed with Janel Scientific Graphing and Statistical Softwares (SigmaPlot/SigmaStat for Windows).

MATERIALS AND METHODS

Rhodopseudomonas sphaeroides S, a non-sulfur purple bacteria was used throughout the experiments. The organism was cultivated on agar

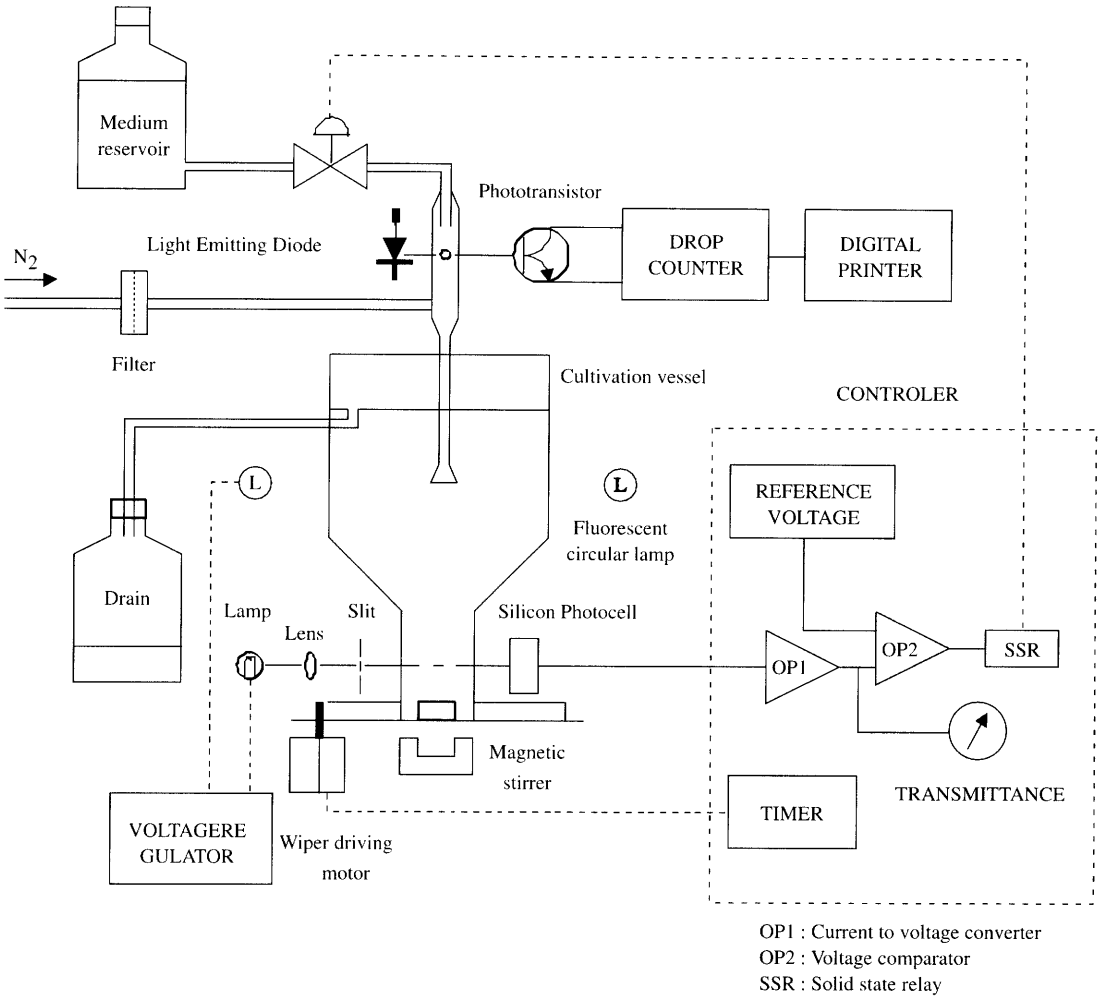


Figure 1 Block diagram of turbidostat culture apparatus.

slant at 30°C for 2 days, stocked at 4°C, and subcultured every week. Medium composition for stock culture was previously described by Sasaki *et al.* (1978). The carbon sources, propionate (C_2H_5COONa, 0-15 g/l) and acetate ($CH_3COONa.3H_2O$, 0-55 g/l) were added to the basal medium (Table 2) either singly or mixed. The mixed carbon sources of preculture medium containing 1.8 g/l of propionate and 0.2 g/l of acetate (Sasaki *et al.*, 1978) were added to the basal medium for inoculum preparation.

Culture apparatus A Turbidostat culture apparatus (Nanba *et al.*, 1983) previously constructed was slightly modified for photoheterotrophical cultivation by placing a circular fluorescent lamp (30 W, FCL 30 BR, 29x225/167 mm, National, Japan) around the 650 ml vessel. The schematic diagram of the whole system is shown in Figure 1.

The mentioned vessel has a lower and upper parts. The upper portion which has a diameter of 90 mm served as a fermentor. The lower portion which has a 28 mm diameter was exposed to a tungsten exciter lamp (L; KE-020, 4 V-0.75 A, Kondo, Silvania Co. Ltd., Tokyo) and the light transmitted through the vessel was absorbed by a silicone-photoelectric cell (PC; Type S 640, Hamamatsu TV Co. Ltd., Shizuoka). The photocurrent corresponding to the bacterial density was converted to voltage by I-V converter. The decrease in the transmittance value due to the increase in bacterial cell was offset by voltage comparator. With this regulation, the solenoid valve was opened to supply the fresh medium. The flow rate of the fresh medium was calculated on the basis of the photoelectrically counted droplet number. This was recorded every 10 min on digital printer (Model DP-104, Nada Electronic Ins. Co. Ltd., Kobe). For the circuit of the control unit, linear operational amplifier (A 3140, R.C.A., USA) was used as an I-V converter and voltage comparator.

Cultivation Pre-cultivation was carried out by inoculating a loop of a week old slant culture of *Rhp. sphaeroides* S to a 50 ml preculture medium in 100 ml test tube with magnetic stirrer. After 1-2 days at 30°C under light intensity of 3,000 lux and microaerobic condition, 10 ml of this preculture suspension was inoculated into 270 ml media having varying concentrations of carbon source. Prior to inoculation, the fresh culture media were adjusted for 100% transmission.

After about half a day of incubation, turbidostat cultures were carried out by controlling light transmission at 90% which was equivalent to a cell concentration of 12 mg dry cell/l. Temperature was maintained at 30±1°C by the heating-cooling system of water circulated tube inserted in the fermentor. Anaerobic condition was maintained by slightly bubbling N_2 at the middle part of the vessel for the continuous outflow of the output culture broth. The light intensity supplied by a 30 W fluorescent circular lamp to the round surface vessel was controlled by the voltage regulator alone or with the strip shaded transparency. When the flow rate of the fresh medium (droplet number per 10 min) became constant for 5 h after 2 days of turbidostat culture, the cultivation was stopped since steady state had been attained.

Analyses

Cell concentration was determined by measuring optical density at 660 nm with a spectrophotometer (Shimadzu UV-200, Kyoto). The linear relationship between OD_{660} and dry weight was prepared from the exponential growth of preculture.

The propionate and acetate concentrations of the supernatant from the steady state culture broth were determined using a gas chromatography (GC-7AG, Shimadzu CO. Ltd., Kyoto). The samples for injection were prepared by first adjusting the pH of the supernatant to 3.0 with diluted HCl solution. The broths were then diluted with deionized water to obtain an appropriate concentration within 1.0 g/l. The operation conditions were as follows:

Detector	a hydrogen ionization detector (FID)
Column packing	Porapak type QS (Water Associates Inc. Co.)
Temperature of column bath	170°C
Temperature of injection port and detector bath	200°C
Flow rate of carrier	

gas (N_2)	50 ml/min
Injection volume	2 µl
Column size	75 cm in length and 3 mm in diameter

At the steady state of the turbidostat, the specific growth rate (µ) is equal to the dilution rate (D). This was determined when the flow rate of the fresh medium (droplet number every 10 min) became constant for 5 h after 2 days of turbidostat culture.

Experimental procedures The optimum conditions for the cultivation of *Rhp. sphaeroides* S under anaerobic-light, pH 6.8 and 30°C were conducted as follows:

(1) *Effects of cell mass on growth rate in propionate medium*

The cell mass was varied at 30, 60 and 90% transmittance giving a cell concentrations of 143, 61 and 12 mg/l, respectively.

(2) *Effects of propionate or acetate and light intensity on growth rate*

C_2H_5COONa and $CH_3COONa.3H_2O$ were varied from 0-15 g/l and 0-55 g/l, respectively. The light intensity was tested at 300, 1,000 and 3,000 lux.

(3) *Effects of mixed salts on the growth rate*

C_2H_5COONa and $CH_3COONa.3H_2O$ were mixed at different concentrations for growth enhancement. To 0, 20, 40, 60 and 80% propionate, 100, 80, 60, 40 and 20% acetate were added accordingly. One hundred percent propionate or acetate corresponded to 5 and 10 g/l, respectively.

(4) *Effects of yeast extract on growth rate*

C_2H_5COONa was decided as a sole carbon source at concentration of 5.0 g/l and the light intensity was at 3,000 lux. The concentrations of yeast extract were 0.5, 1.0, 1.5 and 2.0 g/l.

RESULTS AND DISCUSSION

This work was composed of two parts. The first part the inhibition effects of propionic and acetic acids and the enhancing ability of light on the growth rate of *Rhodopseudomonas sphaeroides S* under anaerobic condition were studied. In addition to this, the optimum concentration of yeast extract and the effect of mixed acids were determined. The second part was on the proposed substrate inhibition models for the determination of kinetic constants.

Optimization of the cultivation condition

(1) *Effects of cell mass on growth rate in propionate medium*

First, the suitable concentration of cell mass was examined for the inhibitory effect of

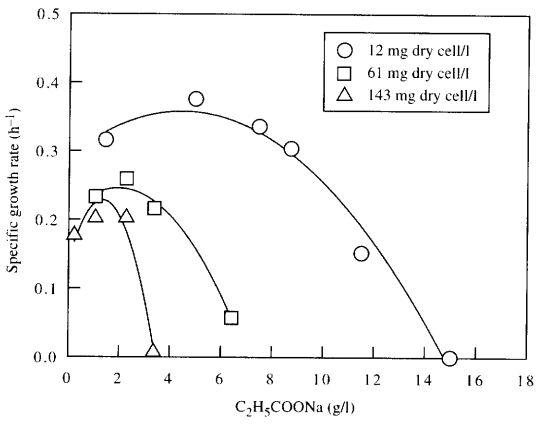


Figure 2 Effects of cell concentration on growth rate of *Rhp. sphaeroides S* in propionate medium at 30°C and 3,000 lux.

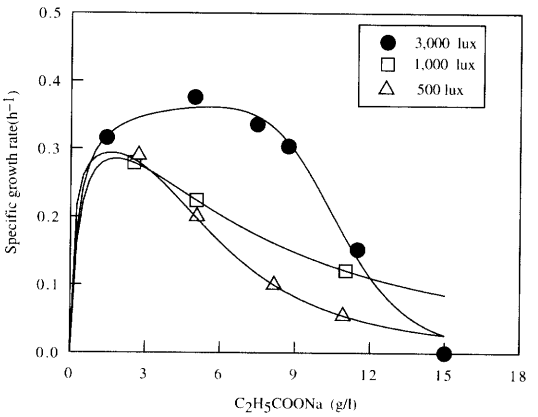


Figure 3 Effects of propionate and light intensity on growth rate of *Rhodopseudomonas sphaeroides S* at 30°C and 12 mg dry cell /l.

propionate on growth rate. Figure 2 shows the results obtained at 143, 61 and 12 mg/l and various concentrations of propionate at 3,000 lux. The increase in cell mass decreased the growth rate, and the concentration of propionate is necessary to inhibit the organism. The possible reason for these findings is that the light energy supplied became inadequate due to the presence of greater cell concentration. With the subsequent experiments, i.e. the effects of propionate and acetate concentrations as well as the light intensity were studied at very low cell concentration of 12 mg dry cell/l.

(2) *Effects of propionate, acetate and light intensity on growth rate*

The inhibitory effects of propionate and acetate on the growth rate were studied at different light intensities under anaerobic condition. The optimum concentration of propionate was 5 g/l under the condition of 3,000 lux of light intensity with specific growth rate of 0.376 h^{-1} (Figure 3). At 3,000 lux, growth rate was strongly inhibited at propionate concentration greater than 10 g/l. Growth was not even observed at 15 g/l propionate. These results implied that this organism has no endurance to noticeably high concentrations of propionate under the condition employed in this experiment. Maruyama and Kitamura (1975) postulated two possible mechanisms for the growth inhibition of *Rhodopseudomonas sphaeroides* S by propionate on glucose medium under aerobic-dark condition. These are the uncoupling of biological energy with cell growth and the inhibition of metabolic pathway.

Acetate was better inhibitory substrate than propionate. Figure 4 shows that a specific growth rate of 0.03 h^{-1} could still be observed even at the maximum concentration (49.4 g/l) of acetate used in the experiment. The optimum concentration of this substrate was 10 g/l and the specific growth rate was 0.373 h^{-1}.

Since this organism is a photosynthetic bacteria, adequate light energy should be supplied during its cultivation. Increasing in light intensity decreased the inhibitory effects of propionate and acetate (Figures 3 and 4). This may implied that at 3,000 lux this organism was somewhat stable lessening the inhibitory effects of these two substrates. For the following experiments, 3,000 lux was used.

(3) *Effects of mixed salts on growth rate*

Maruyama and Kitamura (1975) reported that *Rhodopseudomonas sphaeroides* S

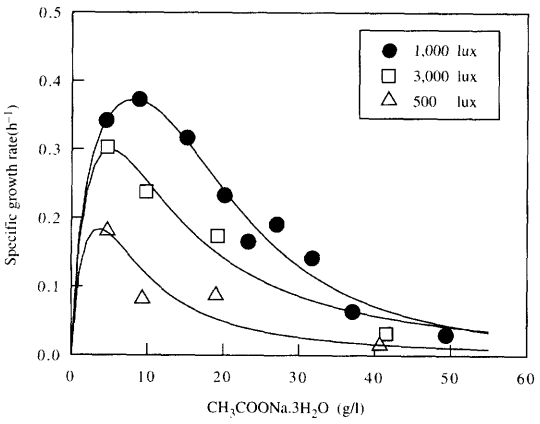


Figure 4 Effects of acetate and light intensity on growth rate of *Rhodopseudomonas sphaeroides S* at 30 °C and 12 mg dry cell /l.

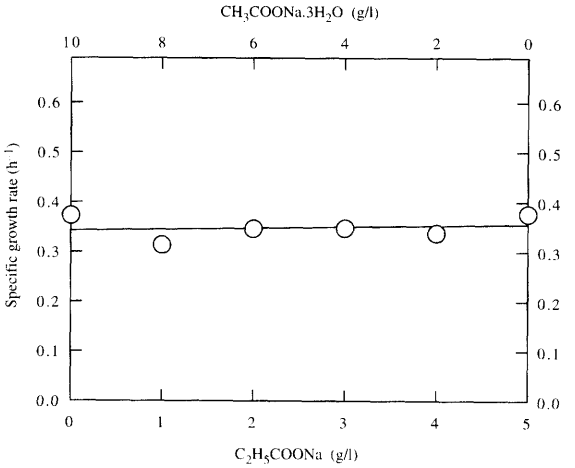


Figure 5 Effects of mixed substrates on growth rate of *Rhodopseudomonas sphaeroides* S at 30°C and 3,000 lux.

grew at a very low rate on propionate medium either in aerobic-dark or anaerobic-light condition. However, with the addition of acetate to propionate medium, it was able to assimilate propionate rapidly. Sasaki *et al.* (1978) also found that the simultaneous utilization of the propionate and acetate slightly increased the maximum growth rate comparing with using propionate alone under aerobic-dark condition. In addition, 67 μg intracellularly accumulated vitamin B_{12} per g dry cell could be obtained by mixing 1.8 g propionate and 0.2 g acetate in 1 l basal medium supplemented with yeast extract and cobalt ion.

In this work, mixtures of propionate and acetate at various concentrations were prepared for growth rate enhancing study. No significant different on the growth rate was observed when both of these substrates were added to the medium (Figure 5). This suggested that the concentration of the mixed salts used were not suitable to enhance the growth rate of the organism under the conditions employed in this experiment. Nevertheless, the specific growth rate was higher compared to 0.h5[1] obtained by Sasaki *et al.* (1978) in propionate-acetate medium and under aerobic-dark condition.

(4) *Effects of yeast extract on growth rate*

It was shown that the addition of 1.5 g yeast extract/l of the medium with CN-B_{12} or cobalt ion accelerated the propionate utilization under aerobic-dark condition (Sasaki *et al.*, 1978). The organic nitrogen compounds in yeast extract might be one of the key factors in propionate metabolism. In this experiment, yeast extract also enhanced the growth rate of the organism at anaerobic-light condition and its optimum concentration was 1.5 g/5 g propionate/l of basal medium (Figure 6).

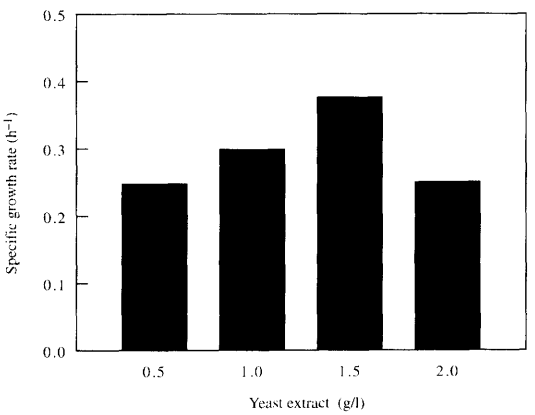


Figure 6 Effects of yeast extract on growth rate of *Rhodopseudomonas sphaeroides* S at 30°C and 3,000 lux.

Determination of kinetic constants using substrate inhibition models

The traditional kinetic models for competitive inhibition by substrate itself can simulate propionic and acetic acids inhibition data. Five empirical equations (Table 1) which have been proposed for growth inhibition by high substrate concentration were considered in this experiment. These functions were fitted to the inhibition data obtained at different concentrations of propionic or acetic acids and different light intensities by nonlinear least squares regression technique.

Among the proposed models (Table 1), models 1 and 3 were found to be suitable for the analysis of the inhibition kinetic of propionic acid. Figure 7 shows the curves obtained by plotting the data from Table 3 using the five proposed models. The following kinetic constants were obtained, at 3,000 lux, $\mu_m = 0.366\ h^{-1}$, $K_S = 2.0$ mM, $K_i = 118$ mM and $n = 8.80$ (Table 4). Comparing the observed data (at 3,000 lux), the values obtained from this model were slightly higher at very high propionic acid concentration. Also, the μ_m obtained from this model was a little lower than μ_m observed. However, its prediction capability to the data at various light intensities tested (3,000, 1,000 and 500 lux) was better than the other models.

Figure 8 gives the graphical comparison of acetic acid inhibition data calculated from five proposed models at 3,000, 1,000 and 500 lux using data from Table 3. The kinetic values computed from these are summarized in Table 5. Just like in propionic acid, models 1 and 3 gave the best satisfactory fit with acetic acid inhibition data obtaining μ_m to be about 0.5 h^{-1}, while μ_m from model 2 gave an unusual higher value (0.764 h^{-1}).

The kinetic constants obtained from models

Table 1 Substrate inhibition models.

Model no.	Function	Remarks	References
1	$\mu = \frac{\mu_m}{1 + \frac{K_S}{C_S} + (\frac{C_S}{K_i})^n}$	Extension of substrate inhibition (Eq. 3)	Takamatsu *et al.*, 1981 Yamane *et al.*, 1976
2	$\mu = \frac{\mu_m}{1 + \frac{K_S}{C_S} + \frac{C_S}{K_i}(1 + \frac{C_S}{K})}$	Substrate inhibition ([ES_2] and [ES_3] are inactive) (Eq. 4)	Edwards, 1970
3	$\mu = \frac{\mu_m}{(1 + \frac{K_S}{C_S})(1 + (\frac{C_S}{K_i})^n)}$	Generalized non-competitive inhibition (Eq. 6)	Edwards, 1970 Nanba *et al.*, 1983 Takamatsu *et al.*, 1981 Yano & Koga, 1973
4	$\mu = \frac{\mu_m C_S \exp^{(\frac{C_S}{K_i})}}{K_S + C_S}$	Modified from product inhibition (Eq. 8)	Edwards, 1970
5	$\mu = \mu_m (\exp^{(-\frac{C_S}{K_i})} - \exp^{(-\frac{C_S}{K_S})})$	Substrate inhibition (Eq. 9)	Edwards, 1970

Table 2 The composition of stock culture and basal media (Sasaki *et al.*, 1978).

Composition	Stock culture medium (g/l deionized water)	Basal medium (g/l deionized water)
Sodium L-glutamate.H_2O	3.8	-
DL-malic acid	2.7	-
Yeast extract	2.0	-
KH_2PO_4	0.5	
K_2HPO_4	0.5	
$(NH_4)_2HPO_4$	0.8	
$MgSO_4.7H_2O$	0.2	
$CaCl_2.2H_2O$	5.3×10^{-2}	
Nicotinic acid	1.0×10^{-3}	
Thiaamine-HCl	1.0×10^{-3}	
Biotin	1.0×10^{-5}	
$MnSO_4.5H_2O$	1.2×10^{-3}	
Ferric citrate	2.45×10^{-3}	
$CoCl_2.6H_2O$	9.5×10^{-4}	
$NaHCO_3$	-	1.0
Agar	15.0	-
pH	6.8	

Table 3 Effects of propionic and acetic acids, and light intensity on the growth rate of *Rhodopseudomonas sphaeroides S*, at 30°C and 12 mg dry cell/l.

Light intensity (lux)	Propionic acid		Acetic acid	
	Concentration (mmol/l)	Specific growth rate (h^{-1})	Concentration (mmol/l)	Specific growth rate (h^{-1})
3,000	13.50	0.316	31.83	0.342
	49.75	0.376	65.86	0.373
	75.28	0.336	108.67	0.317
	94.20	0.304	144.50	0.233
	124.61	0.152	167.34	0.166
	156.22	0.000	193.61	0.191
			226.67	0.142
			265.22	0.064
			353.13	0.030
1,000	26.33	0.279	34.57	0.303
	52.59	0.224	72.92	0.238
	114.77	0.121	142.61	0.174
			307.54	0.032
500	28.36	0.290	35.35	0.181
	52.83	0.200	69.64	0.082
	85.15	0.100	141.21	0.087
	113.78	0.055	301.35	0.015

Table 4 Summary of kinetic results for effects of propionic acid and light intensity on the growth rate of *Rhodopseudomonas sphaeroides S*, at 30°C and 12 mg dry cell/l.

Light intensity (lux)	Model no. (1)	μ_m (h^{-1})	K_s (mM)	K_i (mM)	K (mM)	n	SS
3,000	1	0.366	1.98	117.68	-	8.78	1.91×10^{-3}
	2	0.373	1.13	6.20×10^{5}	0.02		3.51×10^{-2}
	3	0.367	1.99	117.87	-	8.78	1.91×10^{-3}
	4	0.409	1.07	158.52	-	-	4.58×10^{-2}
	5	0.378	5.60	177.04	-	-	4.85×10^{-2}
1,000	1	0.366	3.24	74.46	-	1.60	3.69×10^{-5}
	2	0.373	2.57	155.26	64.26		3.62×10^{-5}
	3	0.366	2.96	76.63	-	1.63	6.06×10^{-5}
	4	0.409	3.05	96.25	-	-	3.55×10^{-5}
	5	0.378	7.68	100.67	-	-	3.50×10^{-5}
500	1	0.366	3.06	58.42	-	2.58	4.08×10^{-7}
	2	0.373	1.21×10^{-8}	2.33×10^{4}	0.12		3.15×10^{-4}
	3	0.367	2.99	59.43	-	2.61	3.14×10^{-7}
	4	0.409	1.29×10^{-6}	66.92	-	-	1.30×10^{-3}
	5	0.378	5.90×10^{-7}	73.01	-	-	2.33×10^{-3}

(1) Based on models in Table 1

Table 5 Summary of kinetic results for effects of acetic acid and light intensity on the growth rate of *Rhodopseudomonas sphaeroides S*, at 30°C and 12 mg dry cell/l.

Light intensity (lux)	Model no. (1)	μ_m (h^{-1})	K_s (mM)	K_i (mM)	K (mM)	n	SS ($\times 10^{-3}$)
3,000	1	0.488	12.96	147.54	-	2.65	3.50
	2	0.764	36.72	7094.04	1.44		4.21
	3	0.496	13.57	150.78	-	2.70	3.49
	4	0.478	2.53	187.54	-	-	12.4
	5	0.410	8.68	220.35	-	-	17.0
1,000	1	0.488	14.85	89.39	-	1.67	1.47
	2	0.764	31.34	63.21	191.94		1.45
	3	0.496	14.69	95.68	-	1.76	1.41
	4	0.478	7.98	133.79	-	-	0.70
	5	0.410	11.69	145.54	-	-	0.81
500	1	0.488	3.80×10^{-7}	19.86	-	1.03	1.65
	2	0.764	70.31	689.89	1.41		3.49
	3	0.496	1.03×10^{-7}	19.33	-	1.02	1.65
	4	0.478	25.52	67.54	-	-	3.57
	5	0.410	19.10	62.27	-	-	4.02

(1) Based on models in Table 1

1 and 3 at different light intensities supported the fact that light energy is necessary for photosynthetic bacteria like *Rhodopseudomonas sphaeroides* S especially at higher concentration of these inhibitory substrates. Thus, with the decrease in light intensity, an accompanying decrease in K_i, an inhibition constant, was observed indicating that the inhibitory effect of propionic and acetic acids, at high substrate concentrations were not so effective when adequate supply of light energy was met. The mechanism of the ability of light to decrease inhibitory effects of these acids is not known. However, the author will try to explain this mode of inhibition based on allosteric effects. According to Dixon and Webb (1979), allosteric effect cause conformational change in the enzyme in such a way that the binding of inhibitor prevented substrate binding or vice versa. In the presence of high substrate concentration, the chances of forming ineffective ES complexes with two or more substrate molecules combined with an active site are greater due to the tendency of the substrate molecules to crowd toward the enzyme. With this, a change in the conformation of the enzyme can lead to unfavorable substrate binding, and finally to inhibition results. With the expose of this enzyme to conditions that would make it stable e.g. greater light intensity, inhibition effect might be eliminated. In the present work, the μ_m obtained from all the light intensities tested, were quite similar.

CONCLUSION AND RECOMMENDATION

An attempt to utilize short chain fatty acids, propionate and acetate as carbon sources by *Rhodopseudomonas sphaeroides S* was considered. A turbidostat culture apparatus (Nanba *et al.*, 1983) modified for photoheterotrophical cultivation was used to attain a steady state after 2 days incubation at 30°C under anaerobic-light condition maintaining extremely low cell concentration (12 mg/l) and desired light intensities (3,000, 1,000 and 500 lux). The most adequate light intensity was 3,000 lux. The optimum concentrations of propionate and

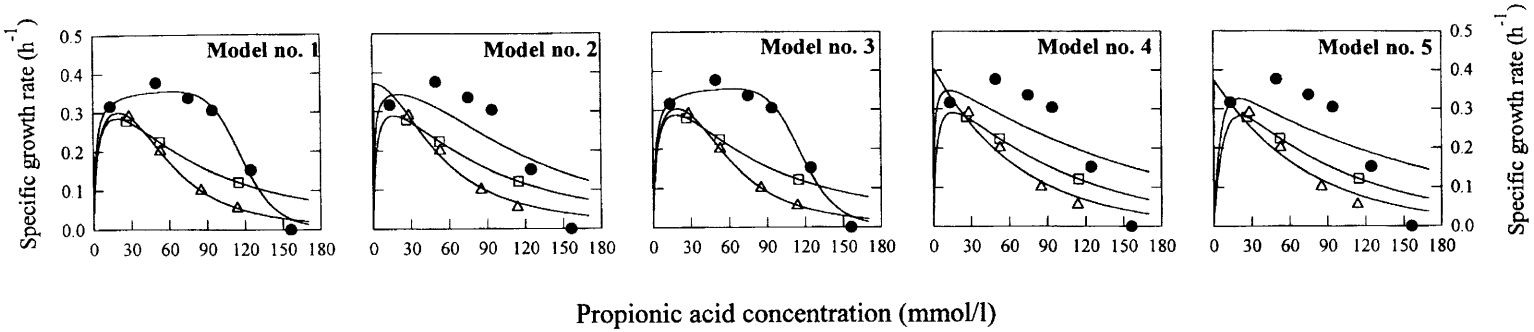


Figure 7 Inhibition modelling of *Rhodopseudomonas shpaeroides S* growing on propionic acid under anaerobic-light condition. (light intensities : ● 3000 lux, 1000 lux, 500 lux; — models; symbol : measurements)

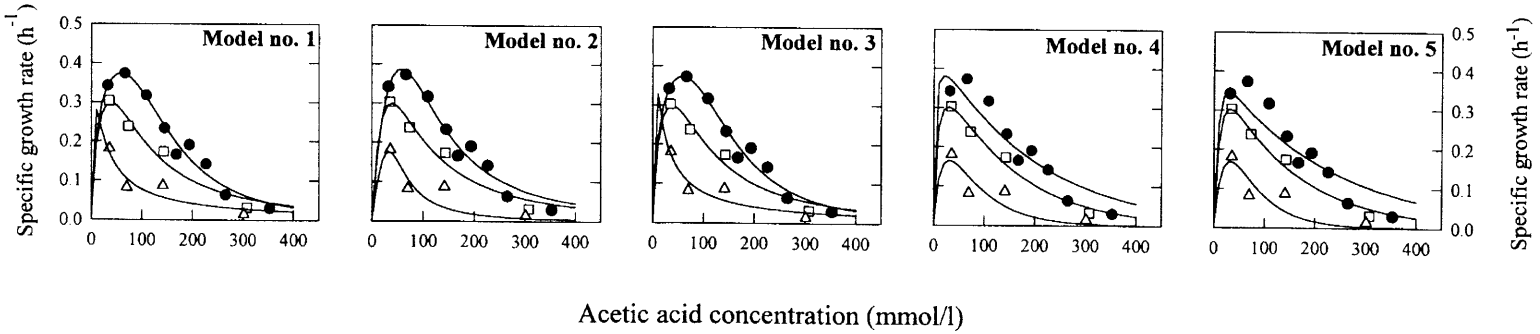


Figure 7 Inhibition modelling of *Rhodopseudomonas shpaeroides S* growing on acetic acid under anaerobic-light condition. (light intensities : ● 3000 lux, 1000 lux, 500 lux; — models; symbol : measurements)

acetate were 5.0 and 10 g/l, respectively. Compared to literature, the specific growth rates (propionate: 0.376 h^{-1}; acetate: 0.373 h^{-1}) at those concentrations were high. These might be due to the low cell concentration used and to the sufficient light energy supplied at 3,000 lux. For propionate, the specific growth rate of 0.03 h^{-1} was still observed at 49.4 g/l. The addition of mixed substrates did not improve the growth rate. The optimum concentration of yeast extract was 1.5 g/l.

Five empirical equations for growth inhibition by high substrate concentration were proposed. These are more complex than those noninhibitory substrates because of the simultaneous fitting of 3 (models 4 and 5) or 4 (models 1-3) kinetic constants. The graphical linear methods can not be easily employed to these kinds of models. Therefore, a statistical method was used to fit the kinetic data to the complex rate equations using a computer. The experimental data were fitted to the models by the nonlinear least squares regression technique. The kinetic constants were estimated by minimizing the sum of squares of the differences between the observed data and the calculated values at each substrate concentration. Among these models, the modified equation of Haldane (1965) and the generalized non-competitive inhibition model were the best because they gave values closed to the data obtained in the experiment. From this model, the K_i values decreased with decrease in light intensity. This suggests that light energy at 3,000 lux was adequate to lessen the inhibitory effect of high concentrations of propionic and acetic acids. The K_i values from the mentioned models were about 118 and 148-151 mM for propionic and acetic acids, respectively. The μm values obtained were about 0.4 h^{-1} and 0.5 h^{-1} for propionic and acetic acids, respectively. The mechanism for the ability of light intensity to reduce the inhibition of organism at high substrate concentration is not known.

Further studies on the effect of other ratios of mixed acids, higher light intensity and low cell mass are recommended. Although models 1 and 3 seem to be the best, there is still a need to formulate a model based on the data obtained in this experiment.

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