Biooxidation of a Synthetic Waste by a Microbial Film Grown on the Liquid Surface in a Shallow Flow Reactor

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ABSTRACT

A new process of biological waste treatment was developed by use of microbial films grown on the liquid surface in a shallow flow reactor. The performance of this process was tested using a synthetic waste that contained acetic acid as a model organic pollutant. About 90% of acetic acid (10,000 mg/L^{-1}) in the synthetic waste was removed by setting $a\ \tau$: (a specific liquid surface area, cm^{-1}, and τ: hydraulic liquid detention time, h) higher than 15 cm^{-1}/h. It was necessary to maintain a large enough (more than 10 cm^{-1} in most cases) to satisfy oxygen demand for the biooxidation. The oxygen balance and TOC determinations showed that the acetic acid removed was completely oxidized with oxygen transferred through the liquid surface. This process would contribute to energy savings, since it requires no forced aeration for oxygen supply.

Index Entries: Microbial film; surface culture; biooxidation of acetic acid; oxygen transfer through liquid surface; *Acetobacter* sp.

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NOMENCLATURE

A	Liquid-surface area, cm^2
a	Specific liquid-surface area, cm^{-1}
C_s	Saturated DO concentration, $g\text{-}O_2/cm^{-3}$ or $g\text{-}O_2/L^{-1}$
E	Dispersion coefficient of waste flow, cm^2/h^{-1}
F	Volumetric flow rate of influent and effluent, cm^3/h^{-1}
H_L	Liquid height of SF reactor, cm
K_s	Saturation constant for acetic acid, g-acetic acid L^{-1}
k_a	Apparent reaction constant, cm/h^{-1}
k_f	Specific rate of acetic acid removal based on liquid-surface area, g-acetic acid cm^{-2}/h^{-1}
k_L	Oxygen transfer coefficient, cm/h^{-1}
k_La	Volumetric oxygen transfer coefficient, h^{-1}
L	Nominal length of open channel type SF reactor, cm
S	Acetic acid concentration of effluent flow, g/L^{-1}
S_i	Acetic acid concentration of influent flow, g/L^{-1}
u	Linear flow rate of liquid, cm/h^{-1}
V	Liquid volume in a reactor, cm^2
Z	Sodium sulfite concentration of effluent, g/L^{-1}
Z_i	Sodium sulfite concentration of influent, g/L^{-1}

Greeks

σ	Standard deviation of residence time of liquid element in reactor, h
τ	Hydraulic liquid detention time in SF reactor, h

INTRODUCTION

In a previous study (*1*), it was observed that the acetic acid production was greatly enhanced by continuous surface cultures of *Acetobacter* sp. in comparison with that of the submerged cutures (*2*). The acetic acid productivity by the liquid-surface culture at the optimum condition was 15× larger than that of the submerged culture. This 15-fold improvement was accomplished only when the liquid depth in the reactor was kept shallow to ensure the high oxygen transfer rate required for the ethanol oxidation.

If microbial film could be developed on the stream of a waste and the organic pollutants in the flow were efficiently oxidized, this shallow flow (SF) reactor process would be promising as a process of saving energy, since it could afford oxygen supply without the forced aeration. According to economics of sewage treatment by conventional activated sludge processes in Japan, some ten billion yen have been spent every year for the electricity for supplying oxygen to the aeration tanks. The present study demonstrates the potential of the liquid-surface culture of bacteria to remove organic pollutants from sewage and industrial waste. The performance of the SF reactor process was tested using a synthetic waste that contained acetic acid as a model organic pollutant.

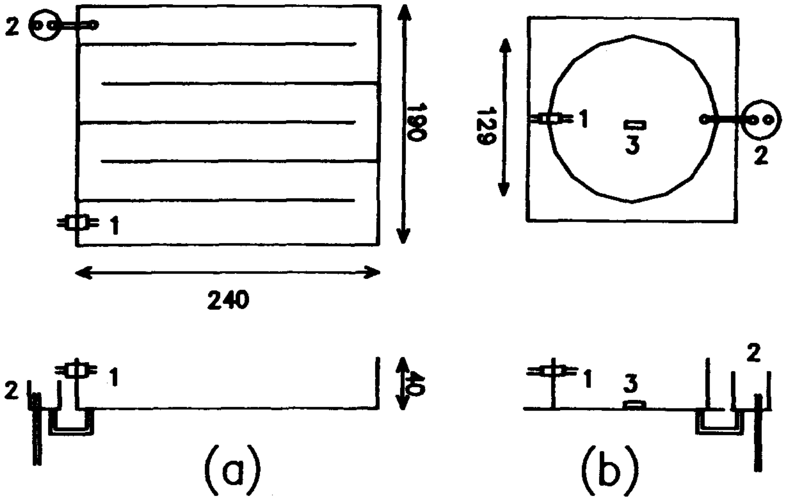

Fig. 1. Shallow flow (SF) reactor for continuous surface culture. (a) Open Channel, (b) stirred vessel; 1, inlet; 2, outlet; 3, stirring rod.

MATERIALS AND METHODS

Bacterial Strain

Acetobacter sp. M7 was used as a seed strain for developing microbial film on a synthetic waste. The stock culture was maintained on an agar slant.

Synthetic Waste

The composition of the synthetic waste used was (in g/L^{-1}) acetic acid 10.0, yeast extract 0.5, peptone 0.2, and glucose 0.1 (pH 3.2). The high acidity of the synthetic waste was suitable for developing selectively microbial film of acetic acid bacteria on the waste.

Shallow Flow Reactor

A schematic representation of the 2 types of shallow flow (SF) reactor is shown in Fig. 1: an open channel reactor with incomplete liquid mixing (Fig. 1a) and a stirred reactor with back-mix flow (Fig. 1b). A thin microbial film was developed on the liquid surface in the reactors according to the methods described later. The liquid in the latter reactor was mixed by rotating a magnetic stirring rod (4 mm in diameter; 15 mm in length) at a relatively slow speed (approx 200 rpm) in order not to break the microbial films on the liquid surface. Most of the experiments were conducted using the open channel reactor, but the kinetic study of the biooxidation of acetic acid was done using the stirred reactor. A small vessel (about 10 mL in vol) was attached to the outlet of the reactors to flow out the effluent without causing mechanical damage to the microbial film in the main reactors. The temperature of the reactors was maintained at 30°C.

Culture

A loop of the stock culture of *Acetobacter* strain M7 was inoculated into a test tube containing 10 mL of the synthetic waste. The preculture was grown overnight at 30°C with shaking, and then mixed into the synthetic waste in reactors. A fresh synthetic waste was fed to the reactor at a linear flow rate less than 3.0 cm/min^{-1}. After approx 1 wk, a thin film of bacteria was observed to be forming on the flowing liquid. Aliquots of the effluent were sampled at appropriate time intervals and subjected to analysis of acetic acid concentration.

Analyses

Acetic acid concentration was determined by gas chromatography (Hitachi 263-50; column, Gaskuropack 54; column temperature, 160°C; injection and detection temperature, 200°C; carrier nitrogen gas flow rate, 35 mL/min^{-1}). Dry weight of microbial film was obtained by drying the film, which was spooned up from the liquid surface and rinsed with tap water, at 105°C to a constant weight.

Oxygen Transfer Rate

Sulfite oxidation method (2) was used to determine the volumetric oxygen transfer coefficient (k_La) of the SF reactor.

Mixing Characteristics of Reactor

A solution of blue ink was fed at the inlet of a reactor as a step input to trace the concentration of the dye at the exit. From the output signal, the dispersion coefficient of the SF reactor was estimated according to eq. (1) (3).

$$(\sigma/\tau) = 2(E/uL) - 2(E/uL)^2(1 - e^{uL/E}) \tag{1}$$

where σ, τ, E, u, and L denote the standard deviation of the tracer curve, the mean residence time of liquid in the reactor, the axial dispersion coefficient, the linear flow rate of liquid, and the reactor length, respectively.

RESULTS AND DISCUSSION

Removal of Acetic Acid in SF Reactor

The acetic acid concentration at the exit of the SF reactor was determined at appropriate time intervals after development of the microbial film. Experimental conditions were varied in the following range: the

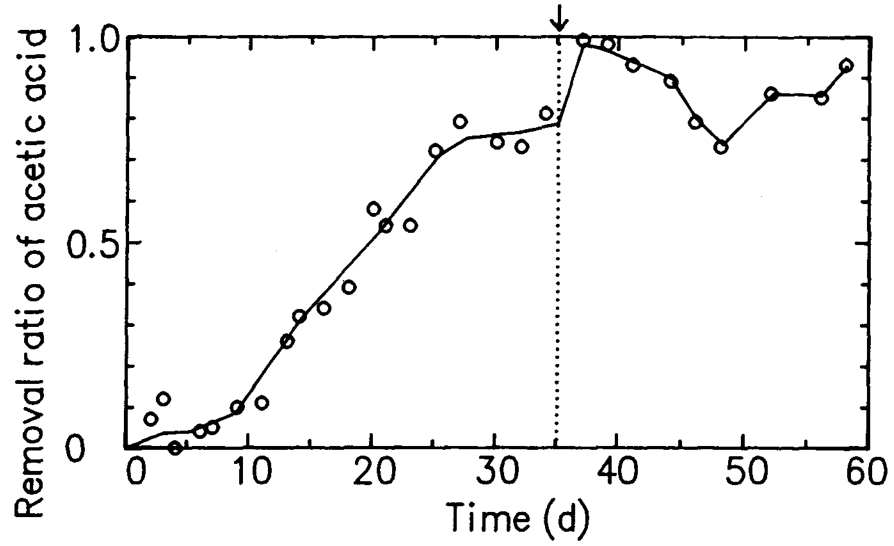

Fig. 2. Time course of acetic acid removal ratio ($1-S/S_i$) by microbial film developed on liquid surface in open channel reactor shown in Fig. 1a. The arrow indicates time of changing hydraulic liquid detention time (τ) from 3.8 to 7.5 h.

liquid height (H_L) = 0.3–2.2 cm and the hydraulic liquid detention time (τ) = 1.5–59 h. Peclet number ($= uL/E$) for liquid mixing in the open channel type SF reactor (Fig. 1a) was in the range of 2.9–18, which indicated that the mixing characteristics of the reactor was neither plug flow nor backmix flow, but incompletely mixed flow.

In an experiment using the open channel type SF reactor of 0.5 cm in liquid depth, the acetic acid concentration in the effluent approached a quasi-steady state within 25 d at $\tau = 3.8$ h and 17 d at $\tau = 7.5$ h, respectively (Fig. 2). With various H_L and τ, the removal ratio ($1-S/S_i$) of the acetic acid concentration at the exit to the inlet concentration was determined, where S_i and S denoted the inlet and the exit acetic acid concentrations. The result is shown in Fig. 3 taking the abscissa as a parametric quantity defined as the product of (the specific liquid surface area) × (the hydraulic detention time of waste), $a\tau$, where a denotes the specific area of the liquid surface in the SF reactor. The removal rate of acetic acid was expressed empirically as follows:

$$dS/dt = -k_a a S \tag{2}$$

where k_a denotes the apparent reaction constant.

The integrated form of eq. (2) gives:

$$1 - S/S_i = 1 - \exp\{-k_a a \tau\} \tag{3}$$

A solid line in Fig. 3 represents eq. (3) where $k = 0.144$ h/cm^{-1}. This numerical value of k_a was obtained from the experimental data by use of the least square method. When the value of $a\tau$ exceeded 15 cm^{-1}/h, about 90% of acetic acid was removed from the synthetic waste. However, it must be

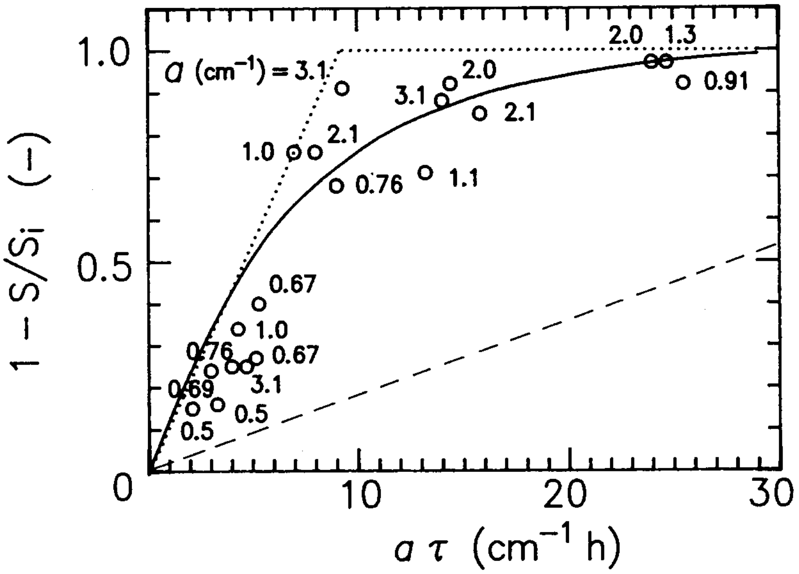

Fig. 3. Correlation of acetic acid removal with $a\ \tau$ in open channel SF reactor. Numbers indicate specific liquid-surface area in cm^{-1}. Solid dotted lines are calculations from eqs. (3), (5) and (8), respectively.

noted that the acetic acid removal was much lower than the prediction from eq. (3), if the specific liquid-surface area was small. For example, Table 1 shows the effect of the specific liquid-surface area on the acetic acid removal in case that $a\tau$ was approx 14. Clearly, the acetic acid removal with the long liquid detention time and the small specific liquid-surface area were inferior to that observed with the short detention time and the large specific area. In the former condition, the oxygen transfer rate through the liquid surface was considered to be insufficient for oxidizing a substantial amount of acetic acid in the reactor so that the long liquid residence time could not improve the acetic acid removal.

Microbial film was also grown on the liquid surface of the back-mix flow reactor (Fig. 1b). The specific rate (k_f) of acetic acid removal per unit of liquid surface area was estimated from the following mass balance at a quasi-steady state:

$$F(S_i - S) = k_f A \tag{4}$$

where F and A denote the flow rate of influent synthetic waste and the liquid surface area, respectively. Eq. (4) is rearranged to give the acetic acid removal:

$$1 - S/S_i = (k_f/S_i)\,(A/V)\,\tau \tag{5}$$

The value of k_f was determined to be 1.12 $mg/cm^{-2}/h^{-1}$ as the mean of 4 experimental runs with different flow rates at fixed liquid volume

Table 1
Effect of Specific Liquid-Surface Area
on Removal of Acetic Acid
in Open Channel Type SF Reactor
($a\tau = 13.2\text{-}14.2\ \mathrm{cm^{-1}/h}$)

a cm^{-1}	τ h	removal %
3.1	4.5	88
1.1	12.0	71
0.83	17.0	54
0.71	20.0	36
0.48	29.0	46

Table 2
Specific Rate (k_f) of Acetic Acid Removal
Based on Liquid-Surface Area in Back-Mix Flow Reactor

run	τ (h)	$1-S/S_i$ (–)	k_f (mg cm^{-2}h^{-1})
1	9.4	0.76	0.81
2	4.7	0.61	1.30
3	4.4	0.54	1.22
4	2.5	0.28	1.13
mean			1.12

(V=131 cm^3) and liquid surface area (A=131 cm^2) (Table 2). The dotted line in Fig. 3 represents the data calculated from eq. (5) where the parametric values of $k_f = 1.12\times10^{-3}$/g/cm^{-2}/h^{-1} and $S_i = 10\times10^{-3}$/g/cm^{-3}. Most of the experimental data of the acetic acid removal are lower than the prediction by eq. (5). This deviation is partly ascribed to the effect of short pass of the liquid in the SF reactor resulting from the incomplete liquid mixing.

Stoichiometric Relationship of Acetic Acid Oxidation

Thirteen aliquots of the effluent samples with different acetic acid concentrations were subjected to TOC determination to obtain a correlation between the acetic acid concentration and TOC of these samples. TOC values determined agreed well with the calculated values assuming the complete oxidation of acetic acid (Fig. 4). This suggests that acetic acid was oxidized almost completely to carbon dioxide, and there remained negligible concentrations of intermediates, if any.

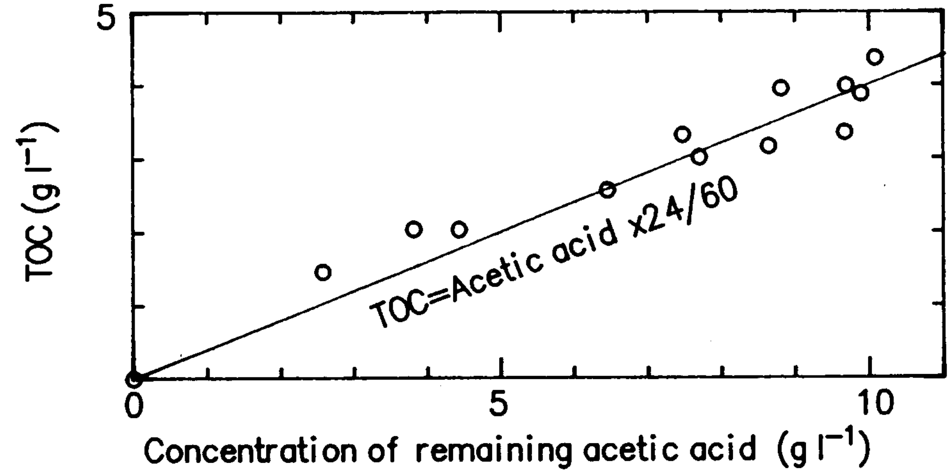

Fig. 4. TOC and acetic acid concentrations of effluent from open channel reactor. Solid line indicates stoichiometric correlation for complete oxidation of acetic acid.

A stoichiometric relationship between acetic acid removal and oxygen consumption was determined using the manometric-type respirometer. Mixrobial film was grown on the liquid surface in the manometric-type respirometer as described in Materials and Methods, and the synthetic waste was passed under the microbial film. Volumetric change of oxygen gas in the head space of the vessel was determined manometrically during the incubation for 30 h at 30°C. The molecular ratio of oxygen consumed to acetic acid removed was $1.6\times10^{-3}\ M/7.7\times10^{-4}\ M=2.07$. This value agreed well with the presumed stoichiometry that 2 mol of oxygen was consumed for the complete oxidation of 1 mol of acetic acid.

The growth yield of bacterial film was estimated based on the integral amount of acetic acid removed: 0.072 ± 0.029 SD g dry matter (g—acetic acid)$^{-1}$ as an average of 5 determinations with different conditions of continuous liquid-surface culture.

The experimental observation described above suggests the following relationship for acetic acid biooxidation: 1 g CH_3COOH + 0.05 g yeast extract + 0.02 g peptone + 0.01 g glucose + 2.2 g $O_2 \rightarrow$ 1.5 g CO_2 + 0.6 g H_2O + 0.072 g microbial film.

OXYGEN TRANSFER CAPACITY OF SF REACTOR

The volumetric oxygen transfer coefficient (k_La) of the SF reactor was measured by the sulfite oxidation method. The amount of sodium sulfite oxidized ($Z_i - Z$) during the detention time (τ) for a sodium sulfite solution

Table 3
Oxygen Transfer Rate (OTR) of Open Channel Type SF Reactor
(by Sulfite Oxidation Method)

run	1	2	3	4
a (cm^{-1})	3.3	1.0	0.67	0.5
τ (h)	3.6	3.9	4.0	4.3
OTR ($g\ l^{-1}\ h^{-1}$)	0.44	0.18	0.10	0.066
OTR/A ($mg\ cm^{-2}\ h^{-1}$)	0.23	0.18	0.15	0.17
k_La (h^{-1})	66	25.8	14.4	12.0
k_L ($cm\ h^{-1}$)	19.8	25.8	21.6	24.0

(50 g/L^{-1}) in the SF reactor was correlated with the volumetric oxygen transfer coefficient (k_La) as shown in the following equation:

$$Z_i - Z = (126/16)\,k_La\,C_s\,\tau \tag{6}$$

where 126/16 is the stoichiometric ratio indicating that 1 mol sulfite is oxidized by 0.5 mol oxygen and C_s, the interfacial oxygen concentration, which is assumed to be equivalent to the saturated oxygen concentration in water. K_La was determined from eq. (6) as $k_La = (16/126)\ (Z_i - Z)/(C_S\tau)$. Effects of the specific liquid-surface area (a) on k_La were investigated by varying the liquid depth of the SF reactor for rather constant values of τ (approx 4 h). Naturally, the value of k_La increased almost proportionally with the increase of a (Table 3). From the proportionality constant, the oxygen transfer coefficient (k_L) was estimated as 20–25 cm/h^{-1} for the oxygen absorption through the liquid surface. In literature, the values of k_L (cm/h^{-1}) are reported for various types of aeration systems: 0.7–14 for the reaeration of a river (*4*); 7–32 (*5*) or 4–14 (*6*) for the oxygen transfer through the free liquid surface of a stirred vessel; 72–180 for surface agitation (*7*); 11–144 (*8*) or 22–180 (*9*) for rising air bubbles; and 108–144 for bubble aeration in a tower fermentor (*6*). However, the oxygen transfer rate determined with the sulfite method was much smaller (about one-fifth) than the theoretical oxygen demand, which was calculated from the amount of acetic acid removed by the bacterial film in the SF reactor at the same hydraulic condition. In a case where oxygen is totally consumed for the biooxidation of acetic acid, the following equation may be derived by assuming that 2 mol of oxygen is used for oxidation of 1 mol of acetic acid:

$$(S_i - S)/t = (60/64)\,k_La\,(C_s - 0) \tag{7}$$

Substituting the numerical values of $k_L = 25\ cm/h^{-1}$: $C_s = 7.6\ mg/L^{-1}$ (in water at 30°C) and $S_i = 10\ g/L^{-1}$ in eq. (7),

$$1 - S/S_i = 0.018\,a\,\tau \tag{8}$$

The data calculated from eq. (8) is shown in Fig. 3 as the broken line. The observed data of acetic acid removal was far greater than that expected from eq. (8). This means that the oxygen absorption rate through the microbial film on the liquid surface was higher than that through the free liquid surface determined with the sulfite oxidation method. The wrinkling surface of the microbial film grown on the liquid surface may enhance the surface area effective for the oxygen transfer in comparison with the smooth surface of a sulfite solution. Since the measurement of the accurate interfacial area of the wrinkled microbial film was not available, it could not be denied that another possibility that the respiratory oxygen consumption in the thin film layer of dense microbial cells on the liquid surface accelerated the physical absorption of oxygen at the air–liquid interface, as suggested by Tsao et al. *(10)* for gas absorption with chemical reaction. It was reported that the interfacial adsorption of motile microorganisms promoted oxygen transfer a few more times more than the rate expected for a uniform suspension *(11)*. Although the microbial film in our experiment was not motile but immobilized on the liquid surface, a similar mechanism of enhancing oxygen transfer might be considered. An investigation in this context will be discussed further in a following report.

In comparison with the biological waste treatment by the microbes fixed on solid particles or rotating disks, the defect of the microbial film on liquid surface is its vulnerability to hydraulic shock. It is necessary to develop a new support, which is a mesh or bush material for fixing the microbial film on the liquid. In the help of the support, the break up of the microbial film at a relatively high liquid flow rate may be avoided.

CONCLUSION

1. A new process for biological waste treatment was developed using surface cultures of *Acetobacter* sp.
2. About 90% of acetic acid in the synthetic waste was removed under the condition that the specific surface area (*a*) exceeded 1.0 cm^{-1} and a product of *a* and the hydraulic liquid detention time was set more than 15 cm^{-1}/h.
3. It was suggested from the oxygen balance and TOC determinations that the acetic acid removed was completely oxidized.
4. The specific rate of acetic acid oxidation on the basis of area of the microbial film was 1.12 $mg/cm^{-2}/h^{-1}$.
5. The rate of oxygen transfer through the microbial film was several-fold larger than that through a free liquid surface.

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