Biodegradation of Long Chain Alkylamines

K. YOSHIMURA, S. MACHIDA and F. MASUDA, Tochigi Research Laboratories, Kao Soap Company, 2606 Akabane, Ichikaimachi, Hagagun, Tochigi 321-34, Japan

ABSTRACT

The relative biodegradability of long chain linear alkylamines has been studied under aerobic condition using the oxygen consumption technique and other methods. Primary and secondary alkylamines with C_4-C_{18} were found to be readily biodegradable. Of the tertiary alkylamines, monoalkyldimethylamines were biodegradable whereas trialkylamines were less biodegradable. Alkylamine-assimilating bacteria were isolated from activated sludge by enrichment culture technique. A strain isolated as PA12 (primary alkylamine)-degrading bacterium was identified as *Pseudomonas putida*. Biodegradability of various alkylamines by this isolated strain also is discussed.

INTRODUCTION

Long chain linear alkylamines and their salts have been widely used as finishing or dyeing agents in the textile industry and as raw materials of cationic or amphoteric surfactants. The increasing amounts of production and use of these alkylamine derivatives make it more important to assess their biodegradability from the ecological point of view.

In a previous paper (1), the biodegradability of long chain quaternary alkylamines was evaluated by determining their inhibitory effect on activated sludge and by comparing the measured oxygen consumption of the alkylamines with their theoretical oxygen demand values. From these results, monoalkyltrimethyl and alkylbenzyldimethyl ammonium chlorides were found to be essentially biodegradable whereas dialkyldimethylammonium and alkylpyridinium chlorides were less biodegradable.

Yamada et al. (2,3) investigated the use of alkylamines by fungi and yeasts. Monoamine-oxidase isolated from *Asperigillus niger* could oxidize primary C_3-C_6 alkylamines to corresponding aliphatic aldehyde and ammonia. Yamada et al. also reported that N-methyl-*n*-butylamine and trimethylamine were demethylated to give *n*-butylamine and dimethyl- or monomethylamine by *Candida* sp. and *Trichosporon* sp., respectively, followed by sequential oxidation to corresponding aliphatic aldehyde and ammonia. However, they did not refer to the use of aliphatic amines with longer alkyl chains.

This paper describes the biodegradability of primary, secondary and tertiary alkylamines with C_4-C_{18} moieties.

EXPERIMENTAL PROCEDURE

Materials

Samples of alkylamines used are shown in Table I. These samples were used as HCl salts.

Biodegradation Tests

Oxygen consumption measurement. Biodegradation tests were carried out with an oxygen consumption measuring apparatus (Ohkura Coulometer) at 25 C. The basal medium was the JIS (Japanese Industrial Standards) BOD diluent prepared by adding 3 ml each of solution A, B, C and D to 987 ml of deionized water (Table II). Thirty or 100 parts per million (ppm) of test alkylamines and 30 ppm of activated sludge were added to the basal medium (3000 ml). Activated sludge was obtained from a municipal sewage plant that treated domestic sewage in Tokyo.

A simple diagram of the automatic oxygen consumption measuring apparatus is shown in Figure 1. The incubation chamber includes an incubation flask with a magnetic stirrer and a small vessel for carbon dioxide absorbent. When dissolved oxygen is consumed by microorganisms and the carbon dioxide generated is absorbed by soda lime, reduction of the inner pressure occurs and the resulting change in the manometric level closes the relay circuit. The oxygen generator then operates and the oxygen is supplied to the incubation flask until the initial pressure is maintained. Since the amount of consumed oxygen is directly proportional to electrolysis time, changes in the millivoltage of electrolysis with time are measured.

Colorimetric, total organic carbon (TOC) and CO_2 production measurements. Colorimetric and TOC measurement techniques were the same as described in a previous work (1); the colorimetric measurement was carried out according to Auerbach's method (Bromophenol Blue) (4), whereas the TOC measurement was with a Beckman TOC analyzer. CO_2 production was measured according to Ito's method (5).

Effect of Alkylamines on the Microbial Glucose Oxidation

Alkylamine (10-100 ppm) and activated sludge (30 ppm) were added to the basal medium containing glucose (40 ppm). The control flask contained no alkylamine. Oxygen

TABLE I

Samples of Alkylamines

Group	Structure	Abbr.
Primary	n-$C_4H_9NH_2$	PA4
	n-$C_8H_{17}NH_2$	PA8
	n-$C_{12}H_{25}NH_2$	PA12
	n-$C_{14}H_{29}NH_2$	PA14
	n-$C_{16}H_{33}NH_2$	PA16
	n-$C_{18}H_{37}NH_2$	PA18
Secondary	$(n$-$C_4H_9)_2NH$	SA4
	$(n$-$C_6H_{17})_2NH$	SA8
	$(n$-$C_{12}H_{25})_2NH$	SA12
Tertiary	$(n$-$C_4H_9)_3N$	TA4
	$(n$-$C_8H_{17})_3N$	TA8
	$(n$-$C_{12}H_{17})_3N$	TA12
	n-$C_{12}H_{25}N(CH_3)_2$	TA1211
	n-$C_{18}H_{37}N(CH_3)_2$	TA1811

TABLE II

Reagents for the Standard Japan BOD Diluent

Reagent	Substrate	g/ℓ Water
A	K_2HPO_4	21.75
	KH_2PO_4	8.5
	$Na_2HPO_4 \cdot 10H_2O$	44.6
	NH_4Cl	1.7
B	$MgSO_4 \cdot 7H_2O$	22.5
C	$CaCl_2$ (anhydride)	27.5
D	$FeCl_3$	0.25

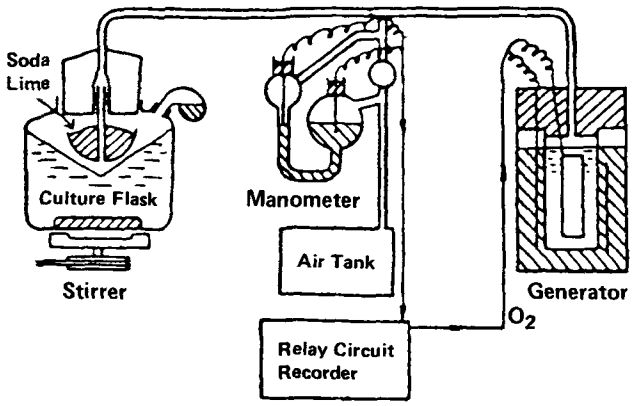

FIG. 1. Schematic diagram of automatic BOD measuring apparatus.

consumption was measured with the same apparatus as already mentioned.

Antimicrobial Activity

The minimal inhibitory concentrations (MIC) of primary alkylamines against 5 bacteria were determined by the Trypic Soy agar-plate method (2 days at 30 C).

Isolation of the PA12 Degrading Bacterium

The PA12 degrading bacterium was isolated in a usual manner from a test flask after acclimation with 30 ppm of PA12. The isolated strain was identified and used for further biodegradation tests, which were carried out at 30 ppm alkylamines.

RESULTS AND DISCUSSION

Effect of Alkylamines on the Microbial Activity

The effect of alkylamines on the microbial glucose oxidation is shown in Table III. Of the primary alkylamines tested at 100 ppm, PA12 showed no oxygen consumption whereas PA8,14 and 16 showed considerable oxygen consumption with slight retardation at the initial stage (1-2 days) of incubation. However, PA12 at 10 ppm did not have an inhibitory effect (Fig. 2). These results were consistent with the tendency of their antimicrobial activity as shown in Table IV. For example, the MIC of PA12 against Gram-positive and -negative bacteria were 10 and 20-50 ppm, respectively. The MIC of PA14 was 50-100 ppm against *Pseudomonas aeruginosa* and *Escherichia coli* and 100-200 ppm against *Proteus vulgaris*. Primary alkylamines except PA12 and PA14 did not inhibit the growth of Gram-negative bacteria even at 300 ppm. Therefore, it is considered that PA12 at 100 ppm showed no oxygen consumption because of its antimicrobial activity.

TABLE III

Effect of Alkylamines on the Microbial Glucose Oxidation

Alkylamine	Relative oxygen consumption[a]
PA4	5.30
PA8	5.30
PA12	0.0
PA14	2.50
PA16	4.36
PA18	2.70
SA4	5.30
SA8	5.30
SA12	1.21
TA4	0.95
TA8	1.28
TA12	1.31
TA1211	2.10

[a]After 7 days (Glc=1.0).

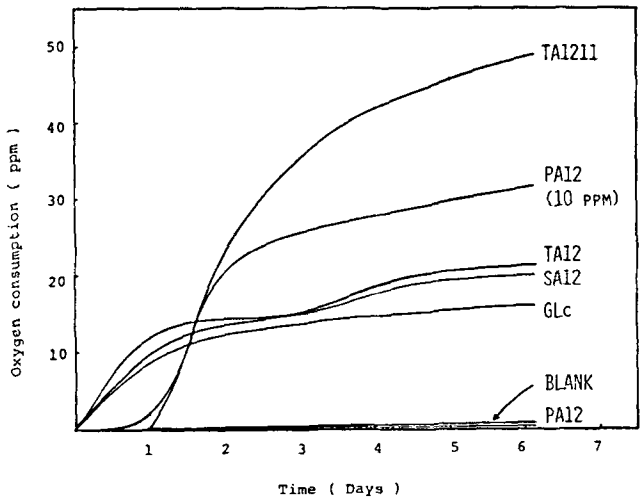

FIG. 2. Effect of PA12, SA12, TA12 and TA1211 on the microbial glucose oxidation (alkylamine 100 ppm, glucose 40 ppm, activated sludge 30 ppm, 25 C).

These antimicrobial activities of PA12 and PA14 may be related to the surface activity and to the solubility. That is, the longer the alkyl chain length of PA salts, the greater the surface activity, but their solubilities (6) become less. In practice, the test solution of PA16 and PA18 at 100 ppm became slightly turbid. On the other hand, secondary and tertiary alkylamines showed no inhibitory effect on the microbial glucose oxidation, but SA8 and TA1211 showed a slight retardation of oxygen consumption at the initial stage. Furthermore, the oxygen consumptions of SA4, 8 and TA1211 were observed to be several times as much as that of glucose alone, which suggested these alkylamines are biodegradable (Table III).

Biodegradability of Alkylamines

The biodegradability curves of primary alkylamines are shown in Figure 3. Primary alkylamines except PA12 exhibited considerable oxygen consumption at 100 ppm. The BOD by theoretical oxygen demand values (BOD/ThOD) of these alkylamines were more than 60% after 12 days. In general, substrates having more than 40% of BOD/ThOD values are regarded as readily biodegradable.

Therefore, these alkylamines are considered to be readily biodegradable. Although PA12 at 100 ppm showed no oxygen consumption, at 30 ppm it consumed oxygen with 1 wk retardation (Fig. 3). Nonbiodegradability of PA12 at 100 ppm is considered to have resulted from its antimicrobial activity (Table III,IV). Figure 4 shows the time course of the BOD/ThOD values of secondary alkylamines at 100 ppm. SA4, 8 and 12 exhibited considerable oxygen consumption and their BOD/ThOD values became more than 50% after 12 days. The alkylamines are considered to be readily biodegradable for these reasons. Figure 5 shows the time course of the BOD/ThOD values of tertiary alkylamines at 100 ppm. TA4, 8 and 12 exhibited only slight oxygen consumption whereas monoalkyldimethylamines, TA1211 and TA1811, exhibited considerable oxygen consumption. Furthermore, Figure 6 shows the time course of CO_2 production, TOC and Bromophenol Blue Active Substance (BPBAS) decrease of TA1211. CO_2 production (%) was represented as CO_2

TABLE IV

Minimal Inhibitory Concentrations (MIC)[a] of Primary Alkylamines for Bacterial Growth

	Gram-positive bacteria		Gram-negative bacteria		
	St. aureus	*B. subtilus*	*Ps. aeruginosa*	*E. coli*	*Pr. vulgaris*
PA8	300<	300<	200-300	300<	300<
PA12	10	10	30- 50	20- 30	20- 30
PA14	30	30	50-100	50-100	100-200
PA16	30	30	300<	300<	300<
PA18	30	50-100	300<	300<	300<

[a]MIC=ppm.

theoretical production (%). BPBAS disappeared and TOC dropped to ca. 50% within 9 days. The tendency of TOC decrease and CO_2 production was consistent with that of oxygen consumption (Figs. 5,6). As CO_2 production increased with the decrease in both BPBAS and TOC, TA1211 was considered to be certainly biodegraded.

The results reported in a previous paper (1), in which monoalkyltrimethyl and alkyldimethyl ammonium chlorides were biodegraded, whereas dialkyldimethylammonium and alkylpyridinium chlorides were not biodegraded, suggest that good biodegradability may be associated with the chemical structure where there are one long alkyl chain and 2 or 3 methyl groups on a nitrogen atom.

On the other hand, nonbiodegradability of trialkylamine TA4, 8 and 12 presumably results from steric hinderance among 3 long alkyl chains on a nitrogen atom.

When PA12 at 100 ppm was mixed with the same concentration of alkylsulfate (SDS), the oxygen consumption of this mixture exceeded that of alkylsulfate alone. PA12 and SDS at 100 ppm correspond to 0.54 mM and 0.36 mM, respectively; PA12 is in excess stoichiometry. It is well known that anionic surfactants form ion pairs with cationic surfactants or alkylamines. Therefore, SDS can neutralize the inhibitory effect of PA12 against bacteria by making an ion pair with PA12. The test medium of SDS at 100 ppm (300 ml) contains 30 mg of SDS.

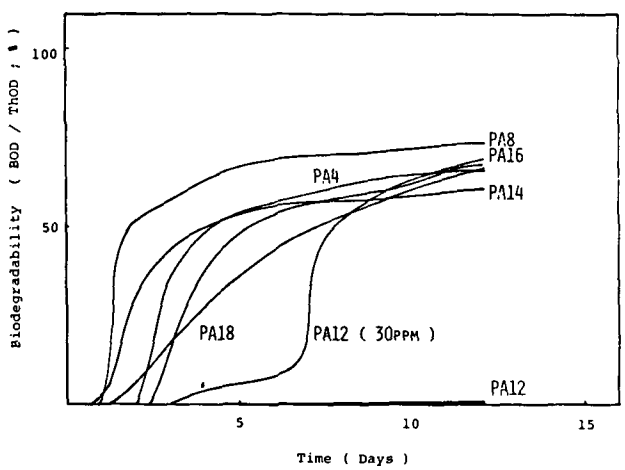

FIG. 3. Biodegradation of primary alkylamines (alkylamine 100 ppm, activated sludge 30 ppm, 25 C).

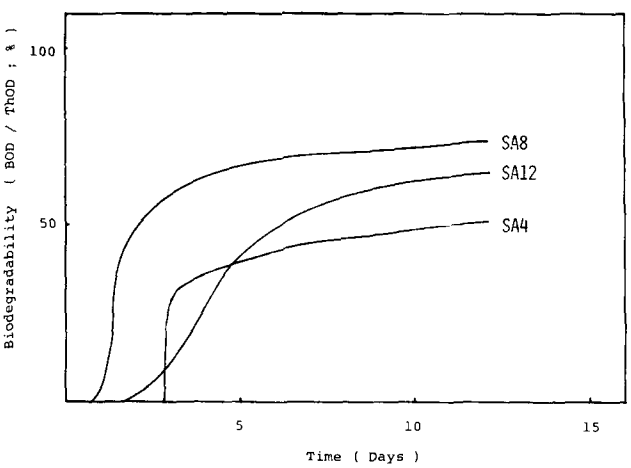

FIG. 4. Biodegradation of secondary alkylamines (alkylamine 100 ppm, activated sludge 30 ppm, 25 C).

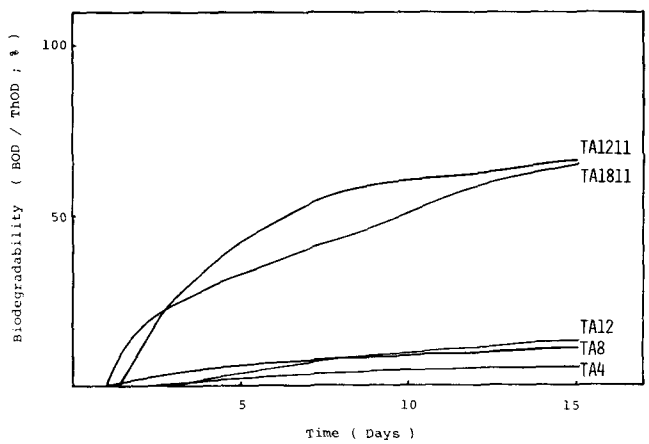

FIG. 5. Biodegradation of tertiary alkylamines (alkylamine 100 ppm, activated sludge 30 ppm, 25 C).

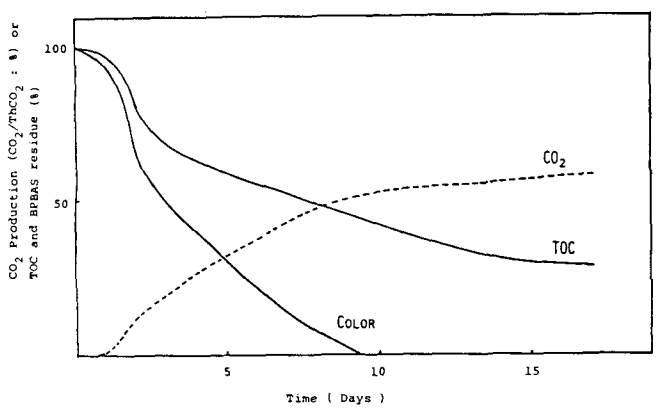

FIG. 6. Biodegradation of TA1211 by CO_2 production, TOC and colorimetric measurement (TA1211 100 ppm, activated sludge 30 ppm, 25 C).

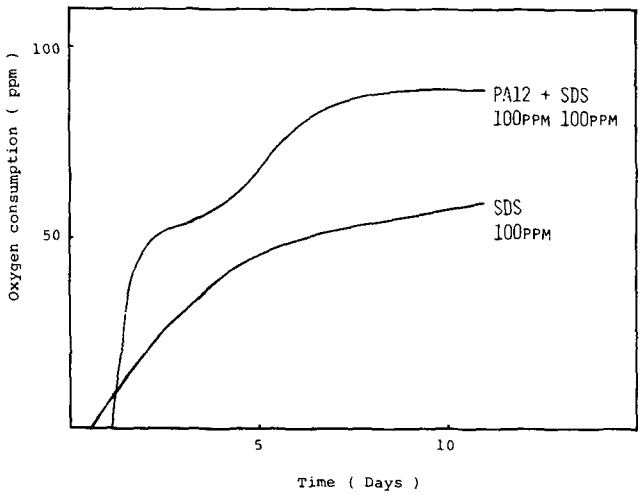

FIG. 7. Biodegradation of PA12 in combination with SDS.

TABLE V

Characteristics of PA12 Degrading Strain

Shape	rod
Mobility	+
Gram's staining	-
Accumulation of poly-β-hydroxy butyrate	-
Fluorescent pigment	+
Pyocyanin	-
Carotenoid	-
Growth at 41 C	-
Oxidase	+
O-F test	oxidation
Dentrification	-
Hydrolysis of gelatin	±
Hydrolysis of starch	-
Assimilation of carbon compounds	+
Glucose	+
Trehalose	±
2-Ketogluconate	+
meso-Inositol	±
Geraniol	-
L-Valine	+
β-Alanine	+
L-Arginine	+

If 30 mg of SDS is completely biodegraded, the amount of oxygen consumption will reach 67.8 mg of oxygen, theoretically. The mixture of PA12 and SDS consumed 89 mg of oxygen (Fig. 7). The excess of this oxygen consumption suggested that both PA12 and SDS were biodegraded. Similar results were reported with cationic surfactant by May (7) and Masuda et al. (1).

TABLE VI

Biodegradation of Alkylamines by Isolated Strain

Alkylamine[a]	BOD/ThOD (%)
PA4	33.6
PA8	46.8
PA12	35.8
PA16	37.3
PA18	32.2
SA8	0.0
SA12	0.0
TA8	0.0
TA12	0.0

[a]Cationic surfactants (monoalkyltrimethyl, alkylbenzyldimethyl and dialkyldimethylammonium chlorides) were not biodegraded.

Isolation and Identification of PA12 Degrading Bacterium

Morphological and physiological properties of isolated bacterium were identical to those of *Pseudomonas putida* as shown in Table V. This isolated strain could degrade PA12 and other primary alkylamines, but not secondary alkylamines, tertiary alkylamines or cationic surfactants (Table VI). Furthermore, this isolated strain could use PA12 as the sole carbon and nitrogen source for its growth. This result suggests the possibility that primary alkylamines are biodegraded through either of these 2 pathways: (a) oxidative deamination by amine oxidase to give the corresponding fatty acid and ammonia (Yamada et al.) (3); or (b) ω-oxidation on the terminal methyl group to give ω-amino fatty acid (Raymond et al.) (5), followed by β-oxidation in either case.

The biodegradation mechanism of these alkylamines is under investigation.

REFERENCES

1. Masuda, F., S. Machida and M. Kanno, VII-International Congress on Surface Active Substances, Section D, 4:129, Moscow, September 12-18, 1976.
2. Yamada, H., O. Adachi and K. Ogata, Agr. Biol. Chem. 29:117 (1965).
3. Yamada, H., O. Adachi and K. Ogata, Ibid., 29:864 (1965).
4. Auerbach, M.E., Ind. Chem. Anal. Ed. 16:739 (1944).
5. Ito, S., S. Setsuda, A. Utsunomiya and S. Naito, Yukagaku 28:59 (1979).
6. Hoerr, C.W. and H.J. Harwood, Ind. Eng. Chem. 44:2923 (1952).
7. May, A. and A. Neufahrt, Tenside Deterg. 13:65 (1976).
8. Raymond, D.D. and M. Alexander, Appl. Environ. Microbiol. 35:935 (1977).

[Received October 1, 1979]