Applied Organometallic Chemistry (1989) 3 249–255

0268-2605/89/03307249/$03.50

Biodegradation of bis(tri-n-butyltin) oxide

Tian Shizhong,* Y K Chau† and D Liu
National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ontario, Canada L7R 4A6

Received 15 August 1988 *Accepted 26 November 1988*

Bis(tri-n-butyltin) oxide can be biodegraded by a mixed bacterial culture from activated sludge in cyclone fermentors under aerobic and anaerobic conditions with half-lives of five and three days respectively. The degradation follows a sequential dealkylation process. All the intermediates and end products are determined in the degradation process for mass balance calculation. Degradation under different nutrient conditions has been investigated. Adsorption losses for each of the butyltin and inorganic tin species on container walls have also been assessed and discussed.

Keywords: Biodegradation, aerobic degradation, anaerobic degradation, Bis(tri-n-butyltin) oxide, tributyltin, dibutyltin, monobutyltin, inorganic tin

INTRODUCTION

Tributyltin compounds (TBT) are mainly used as industrial biocides. Their use in antifouling paints on ships, boats and docks has caused concern because of the release of the highly toxic tributyltin species (Bu_3Sn^+) into the aquatic environment. Bis(tri-n-butyltin) oxide (TBTO) is the most commonly used compound for these purposes. Biodegradation of tributyltin species under aerobic conditions has been documented;[1–6] however, anaerobic degradation of TBTO by *Pseudomonas aeruginosa* was observed not to be successful.[7] Few studies have been conducted on the anaerobic degradation of TBTO.[7,9]

Some of the TBTO degradation studies were based on the concept of primary biodegradation, i.e. following the disappearance of the parent compound in the test system. Such an approach has certain drawbacks. A typical source of error that may arise is adsorption of the test chemical by reaction vessels, leading to a false conclusion regarding degradation. This paper describes our studies on microbial degradation of TBTO through quantitative recovery of its biotransformation metabolites and degradation end products. In addition, factors such as nutrient availability and aerobic/anaerobic conditions, which may influence a chemical's persistence in the environment, were also studied in an attempt to delineate the pathways and fate of TBTO in the aquatic environment.

MATERIALS AND METHODS

Bis(tri-n-butyltin) oxide (TBTO), di-n-butyltin chloride, n-butyltin chloride, tin(IV) chloride, and ethylmagnesium bromide were obtained from Ventron (Danvers, MA, USA). Tropolone was obtained from Aldrich (Milwaukee, WI, USA). Yeast extract was from Difco (Detroit, MI, USA). All solvents were pesticide grade from Caledon (Georgetown, Ont., Canada).

The TBTO standard solution was prepared by dissolving an appropriate amount of TBTO in methanol to give a solution of 1.0 mg cm^{-3} as tin.

Fresh municipal sludge solution was obtained from the Burlington, Ontario, sewage treatment plant. The activated sludge has a typical mixed liquor suspended solid (MLSS) concentration of 1900 to 2400 mg dm^{-3}.

All-glass cyclone fermentors[10] were used in the biodegradation study. Eight fermentors were generally used in the experiments; four were operated under aerobic, and the other four under anaerobic, conditions. Each fermentor was charged with 1.5 dm^3 of Lake Ontario water to which 1.5 cm^3 of the TBTO standard solution (1.0 mg Sn cm^{-3}) and 10 cm^3 of the fresh municipal sludge was spiked. The final concentration of TBTO was 1.0 mg dm^{-3} expressed as tin. In each set, one fermentor was used as a control which contained TBTO and a microbial growth inhibitor (mercuric chloride at 10 mg dm^{-3}) without addition

*Department of Environmental Science, Wuhan University, People's Republic of China.
†Author to whom correspondence should be addressed.

of the activated sludge. All experiments were carried out at 20 ± 1 °C.

For the investigation of the effects of nutrient level on the rate of degradation, varying concentrations (final concentrations of 0.33 and 3.33 g dm^{-3}) of a nutrient solution consisting of peptone, glucose, sodium acetate and yeast extract (2:2:2:1, by wt), were added to the six fermentors containing 1.5 dm^3 of lake water and 1.5 cm^3 of TBTO solution. The two controls contained no nutrient. The fermentors were purged with a flow of air (aerobic) or nitrogen (anaerobic) at about 20 cm^3 min^{-1}, and the flows were maintained for the whole experimental period.

In the degradation study, a 5 cm^3 sample was taken from the fermentor at 0, 3, 6, 13, 17, 21 and 26 days for analyses of the butyltin species and inorganic tin according to a method given below. The half-life ($t_{1/2}$) of TBTO was determined graphically by plotting the percentage of the remaining test chemical in the fermentor broth against time.

Determination of butyltin species in the fermentor solution

A modified tropolone extraction and Grignard derivatization technique[11] was used for the determination of butyltin species in the fermentor solution. In this method, the butyltin species and tin(IV) in 10 cm^3 of water sample were extracted with 5 cm^3 of tropolone–benzene solution (0.5 %) at pH 1–2. Ethylmagnesium bromide (0.2 cm^3) was used for derivatization of the extracted butyltin and tin(IV) species. The resulting derivatives were ethylbutyltins and tetraethyltin from which the original forms could be recognized. The rationale of using ethyl derivatization was to allow detection of the methyltin species which may occur in natural samples. A silica gel (containing 3 % water) column was used to remove the excess tropolone. The derivatized butyltin compounds were eluted with 30 cm^3 of hexane. After reduction of hexane volume to 0.5 cm^3 in a rotary evaporator, a suitable aliquot was injected to the gas chromatograph–atomic absorption spectrometer system (GCAA) for the determination of the butyltin and tin(IV) species. In the GCAA chromatograms, the ethylbutyltin derivatives elute in the order of their molecular weights with the following retention times in minutes: Et_4Sn (1.95), Et_3BuSn (3.19), Et_2Bu_2Sn (4.49), $EtBu_3Sn$ (5.69). TBTO was determined as the Bu_3Sn^+ species. The detection limit of the method for water was 5 ng dm^{-3} as tin.

The GCAA system used has been described in a previous publication,[9] except that in the present case a 30 m fused silica Megabore column, with a 1.5 m film thickness of 100 % methyl polysiloxane coating (J&W Scientific, CA, USA) was used in place of the packed column. The Megabore column gives sharper peaks, shorter retention time and enhanced sensitivity.

RESULTS AND DISCUSSION

Biodegradation of TBTO under aerobic and anaerobic conditions

The role of micro-organisms in the degradation of tributyltin in the natural environment is not fully understood, although certain strains of micro-organisms, capable of degrading sublethal amounts of TBT during aerobic growth in the presence of a suitable carbon source, have been isolated.[7] Other marine and freshwater micro-organisms and fungi have also been reported to degrade TBT. A summary of the more recent degradation studies is listed in Table 1. In all these studies, the degradation products were mostly the dibutyltin and monobutyltin species. The end product of degradation, the tin(IV) species, was either not reported or not determined, and the half-life of degradation was estimated by extrapolation from the disappearance of TBT in the test solution. No mass balance calculation of the degradation products, or estimation of the adsorption loss, were taken into account. In a preliminary study,[9] a half-life of eight days was reported for the degradation of tributyltin chloride under anaerobic conditions in a similar cyclone fermentor, using sediment extract as inoculum in tap-water medium. The experimental period, however, lasted only for eight days, which was barely sufficient to estimate the half-life of anaerobic degradation.

Laboratory biodegradation of TBTO using an activated sludge inoculum in a fermentor without organic nutrients supplement indicated that TBTO degraded to dibutyltin (Bu_2Sn^{2+}) and tin(IV) in three days (Figs 1A, 1B). The degradation appears to proceed through a sequential dealkylation process. Under anaerobic conditions, substantial amounts of tin(IV) were present in the fermentor solution compared with the trace

Table 1 Summary of research on biodegradation of tributyltin by micro-organisms

Organism	Conditions	Degradation products	Half-life[a]	Ref.
P. aeruginosa	Aerobic, 30°C peptone glucose	Bu_2Sn^{2+}, $BuSn^{3)}$	nd	7
C. puteana, T. versicolor	Aerobic, 30°C	Bu_2Sn^{2+}, $BuSn^{3+}$	nd	7
C. puteana, S. brinkmanii, C. versicolor	Aerobic, 37°C, 1 h, mineral salts sawdust medium	Bu_2Sn^{2+}, $BuSn^{3+}$	nd	1
Chesapeake Bay micro-organisms	Aerobic, 28°C, *in situ*	Bu_2Sn^{2+}, $BuSn^{3+}$	nd	2
Marine and estuary waters	Aerobic, 12–28°C, *in situ*	Bu_2Sn^{2+}, $BuSn^{3+}$	7–15 d	3
Fresh water	Aerobic, 20°C	Bu_2Sn^{2+}, $BuSn^{3+}$	6 d	4
Marine and estuary waters	Aerobic, *in situ*, high chlorophyll	Bu_2Sn^{2+}	2 d	5
Harbours and estuary systems	Aerobic, water column	Bu_2Sn^{2+}, $BuSn^{3+}$	5–14 d	8
Harbour system	Aerobic, 20°C	$BuSn^{3+}$	5.5 months	8
Harbour water spikes	Aerobic, 20°C	Bu_2Sn^{2+}, $BuSn^{3+}$	10 d	8
Sediment water system	Aerobic, 20°C	Bu_2Sn^{2+}, $BuSn^{3+}$, Sn(IV)	4 months	6
Sediment extract, tap-water	Anaerobic, 20°C cyclone fermentor	Bu_2Sn^{2+}, $BuSn^{3+}$, Sn(IV)	8 d	9
Activated sludge, lake water	Aerobic, 20°C, cyclone fermentor	Bu_2Sn^{2+}, $BuSn^{3+}$, Sn(IV)	5 d	This work
	Anaerobic, 20°C, cyclone fermentor		3 d	This work

[a] Abbreviations: nd, not determined; d, days.

amounts under aerobic conditions. Degradation of TBTO to inorganic tin occurred more efficiently under anaerobic conditions. The sequential degradation rates to allow each product to degrade further must also be faster, judging by the presence of large quantities of inorganic tin in the system, under anaerobic conditions. Simultaneously, the half-lives of the intermediate compounds under anaerobic condition must accordingly be shorter.

In biodegradation studies, much attention is often paid to aerobic degradation. There is frequently a failure to appreciate the importance of the anaerobic environment under which some micro-organisms may carry out their biodegradation process more efficiently. For example, it has been reported that the pesticide fenitrothion (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl thiophosphate)[10] and the industrial chemical 2,4-dinitrotoluene[12] were both biodegraded remarkably faster under anaerobic conditions.

In a 26-day degradation experiment (Figs 2A, 2B), the half-lives for degradation of TBTO under natural conditions with no nutrient enrichment, as estimated from the slopes of the degradation curves, were found to be three and five days respectively for anaerobic and aerobic conditions. The initial degradation rates are quite different for the two systems in the first 10 days. For example, degradation rates for 1 mg dm^{-3} of TBTO were 17 % day^{-1} and 10 % day^{-1} respec-

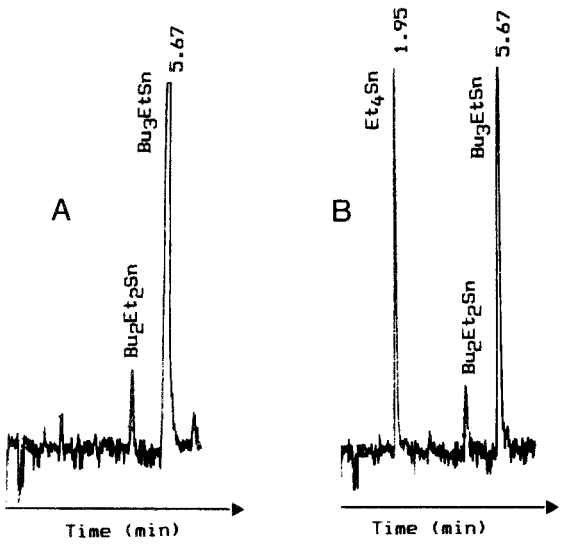

Figure 1 Chromatograms showing biodegradation of TBTO in a fermentor after three days without added nutrients: (A) aerobic; (B) anaerobic.

tively under anaerobic and aerobic conditions in the first five days. After the initial degradation, the rates levelled off and were quite similar at *ca* 1.2 % day^{-1} for both systems from the tenth day onwards.

In order to assess whether the disappearance of TBTO in the biodegradation system was partially due to adsorption losses on the surface of the fermentor walls without degradation, recovery of all the tin species was carried out after completion of the experiments. The empty fermentors were individually extracted twice with 50 cm^3 of tropolone solution and 400 cm^3 of distilled water at pH 1–2. The tropolone extracts were combined and concentrated in a rotary evaporator, derivatized with the ethyl Grignard reagent, cleaned-up in a silica gel column, and analysed for the various butyltin and tin(IV) species. Results tabulated in Table 2 indicated that the recoveries under anaerobic conditions were satisfactory. Under aerobic conditions, recoveries were relatively low (*ca* 50 %). It is possible that under the latter conditions, tin(IV) tends to form insoluble oxides and hydrous oxides which are not extractable by tropolone, giving rise to low recovery of the tin(IV) species in the solution and container. Granting that these reasons for low recovery are true, which could lead to falsely high degradation results, degradation under aerobic conditions was still not as effective as that under anaerobic conditions. If the low recovery was due to loss of TBT to container walls, this would make the actual aerobic half-lives even greater than those indicated in Table 2. All the possible scenarios support the conclusion that degradation is more effective under anaerobic conditions, as was shown by the earlier study.[12]

From the material balance data, it is indicated that, although there is adsorption of TBT and tin(IV) species on the container walls, there is evidence of TBT degradation in the fermentor solution. In the anaerobic degradation without nutrient addition, adsorption on container walls was observed. The adsorbed tin species was mainly tin(IV), not TBT, however.

The half-life values for TBT obtained in this study

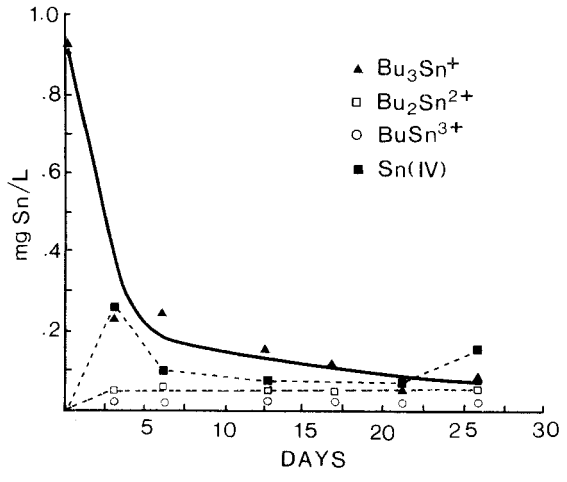

Figure 2A Biodegradation of TBTO under anaerobic conditions. L, litre (dm^3).

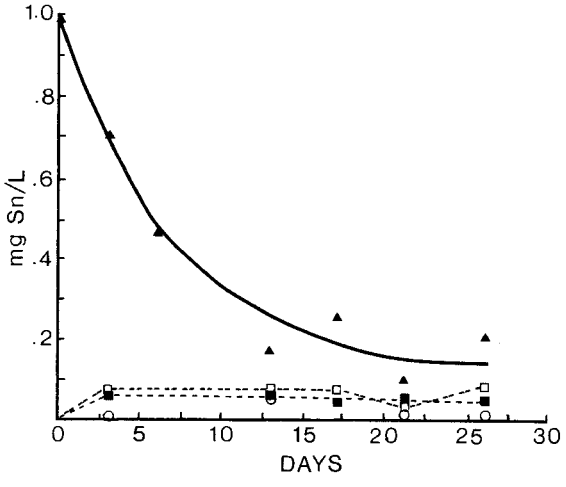

Figure 2B Biodegradation of TBTO under aerobic conditions. Symbols as in Fig. 2A; L, litre.

Table 2 Recovery of butyltin and inorganic tin(IV) species after degradation[a]

Condition	Phase	Tin(IV)	$BuSn^{3+}$	Bu_2Sn^{2+}	Bu_3Sn^+	Total tin	Recovery[b]
Anaerobic, no nutrient	Solution	0.16	nd	0.06	0.10	0.32	0.88
	On wall	0.47	0.02	nd	0.07	0.56	
Anaerobic, nutrients 0.33 g dm^{-3}	Solution	0.10	0.03	0.04	0.35	0.52	0.78
	On wall	0.07	0.01	0.01	0.17	0.26	
Aerobic, no nutrient	Solution	0.05	0.01	0.08	0.21	0.35	0.45
	On wall	0.01	0.01	nd	0.08	0.10	
Aerobic, nutrients 0.33 g dm^{-3}	solution	0.02	nd	nd	0.27	0.29	0.49
	On wall	0.004	0.004	0.004	0.19	0.20	

[a] TBTO spike = 1.00 mg Sn dm^{-3}; concentrations are expressed in mg Sn dm^{-3}.
Abbreviations: nd, not detectable. Analyses were carried out at the end of the 26-day experiment.
[b] Recovery × 100 = percentage.

were shorter in comparison with the reported values (Table 1). The differences in experimental conditions and nutrient substrates are probably the factors responsible. The cyclone fermentor is an effective system with the growth medium running at fast speeds (15.4 dm^3 min^{-1}), creating enormous thin film surfaces for biological activities, simulating more closely the river conditions than does a still-water sysem or the conventional shaker systems commonly used in most laboratories.

Effects of nutrient concentration on degradation rate

The importance of co-metabolism in TBTO biodegradation was also examined. Addition of organic nutrients to the medium was found to cause a dramatic increase in bacterial population, but it did not speed up the degradation of TBTO under both aerobic and anaerobic conditions. On the contrary, the degradation was slowed down (Figs 3A, 3B). Under anaerobic

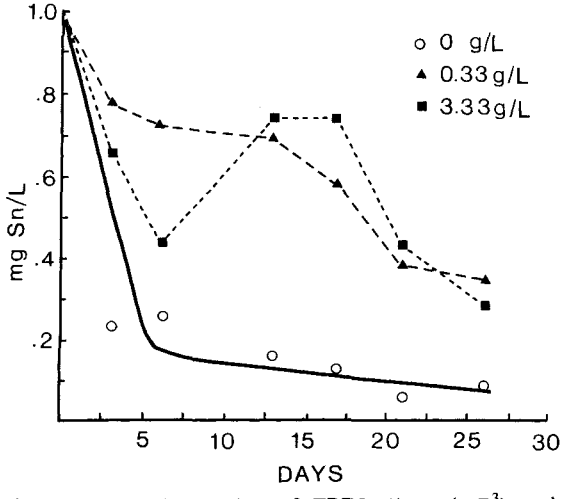

Figure 3A Biodegradation of TBTO (1 mg dm^{-3}) under anaerobic conditions and different nutrient levels. L, litre.

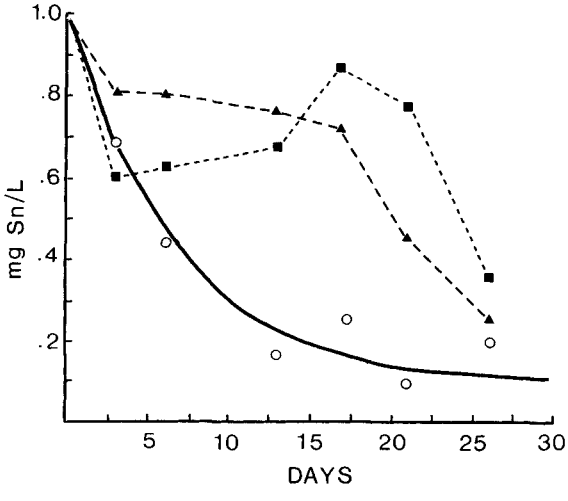

Figure 3B Biodegradation of TBTO (1 mg dm^{-3}) under aerobic conditions and different nutrient levels. Symbols as in Fig. 3A; L, litre.

conditions, the half-life was three days without nutrient addition, versus a half-life of *ca* 20 days when 0.33 g dm^{-3} and 3.3 g dm^{-3} of nutrient were added. The slowing of the degradation was probably due to the abundance of nutrient available to the micro-organisms, and consequently the assimilation of carbon from TBT through a dealkylation series became less preferred. However, when nutrient is limited in the biodegradation system, the micro-organisms will be forced to utilize all available carbon sources, including TBT, for energy and growth. This is a common phenomenon in microbial degradation of persistent chemicals. Glucose was found to suppress the rate of pentachlorophenol degradation for the same reason.[13]

Biodegradation process and the control

Controls in biological experiments are necessary, in view of the complications and artifacts that may occur in the processes. However, sometimes controls designed for a biological purpose introduce other effects to the chemical system. Replicate controls were prepared in parallel by putting into the fermentors the same volume of lake-water spiked with the same quantity of TBT, but containing a biocide (1000 mg dm^{-3} chloroform or 10 mg dm^{-3} mercuric chloride) to inhibit microbial growth. The purpose of the control was to provide data to discriminate any other degradation processes which were not of a biological nature. Unfortunately data from several controls did not provide clear-cut results. The controls also showed somewhat of a decrease of tributyltin concentration, but with no significant amounts of the degradation products to substantiate degradation. It was decided that further studies should be carried out to investigate whether the loss of TBT in the control fermentor was due to adsorption, chemical interactions with the biocides used or chemical degradation.

Chemically, neither chloroform nor mercuric chloride was found to interfere with the TBT determination. Washing the control fermentor walls with tropolone solution and diluted acids did not recover any quantities of TBT to account for the loss. A trap containing glycerol–methanol placed at the top vent of the control fermentor did not show any TBT volatilized from the solution. Thus the loss of TBT was not due to any of these suggested routes. The only difference between the control and the degradation systems was the addition of chloroform or mercuric chloride to the control. Degradation products dibutyltin, monobutyltin and tin(IV) were present in experimental fermentors but not the control which, in the case of anaerobic degradation, made up a mass balance of over 80 %. Consequently, we felt that biodegradation data based on the concept of primary degradation must be used with caution, particularly when such data are extrapolated to predict the fate of a chemical in the natural environment. An approach using the combination of primary degradation and ultimate degradation (i.e. following the formation of intermediates, metabolites and end products) provides more convincing evidence to support the biodegradation of TBTO in the fermentor system, although the aerobic control did not perform as expected in its material recovery.

Surface agar plating of the fermentor solutions was also performed to determine the microbial populations. It was observed that all the control fermentors remained sterilized during the experimental period, whereas intense bacterial growth was observed in the degradation fermentors. This provided evidence that microbial activity was involved in the degradation process.

From these data, we conclude that micro-organisms present in the activated sludge were responsible for degradation. This study also reiterates the fact that disappearance of a compound in the test system without accountable degradation products cannot be taken as quantitative evidence for degradation.

REFERENCES

1. Orsler, R J and Holland, G E *Int. Biodeterioration Bull.*, 1982, 18: 95
2. Olsen, G J and Brinckman, F E *Proc. Oceans 1986 Conference and Exposition, Washington, DC*, vol 4, p 1196
3. Seligman, P F, Valkirs, A O and Lee, R F *Proc. Oceans 1986 Conference and Exposition, Washington, DC*, vol 4, p 1189
4. Thain, J E, Maldock, M J and Waile, M E *Proc. Oceans 1987 Conference and Exposition, Halifax*, vol 4, p 1398
5. Lee, R F *Soc. Environ. Toxicol. & Chem. 8th Ann. Meeting, 1987*, no. 326 (abstract)
6. Maguire, R J and Tkacz, R J *J. Agric. Food Chem.*, 1985, 33: 947
7. Barug, D *Chemosphere*, 1981, 10: 1145
8. Seligman, P F, Grovhoug, J G, Stang, P M, Valkirs, A O, Stallard, M O and Lee, R F *Preprint Extended Abstract, ACS Div. Environ. Chem., Toronto*, 1988, p 573
9. Maguire, R J, Liu, D L S, Thomson, K and Tkacz, R J NWRI Contribution No. 85-82, 1985

10. Liu, D, Strachan, W M J, Thompson, K and Kwaniewska, K *Environ. Sci. & Technol.*, 1981, 15: 788

11. Chau, Y K, Wong, P T S and Bengert, G A *Anal. Chem.*, 1982, 54: 246

12. Liu, D, Thomson, K and Anderson, A C *Appl. Environ. Microbiol.*, 1984, 47: 1295

13. Liu, D, Thomson, K and Strachan, W M J *Bull. Environ. Contam. Toxicol.*, 1981, 26: 85