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Biotransformation of Tributyltin Chloride in the Presence of Resting-Cell Suspensions of Pure Strains of Microorganisms

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We describe the degradation of tributyltin chloride by several strains of fungi, yeasts and bacteria under resting-cell conditions in phosphate buffer, with low initial concentrations of substrate. Yields of biotic conversion of tributyltin ranging from 10 to 77% were observed after a five-day incubation at 28 °C. In most cases, dibutyltin and monobutyltin compounds and a fraction of volatile products were formed. Volatile tin compounds essentially included derivatives of monobutyltin and traces of other organomethyltins (mono-, di-, and trimethyltins; di- and tri-butyltins), probably as the corresponding organostannanes. Compared with conditions in which the substrate was incubated with growing microorganisms, higher yields of degradation and substantial amounts of volatile products were obtained.

Keywords: biotransformation; butyltin; microorganisms

INTRODUCTION

Trisubstituted organotin compounds are often found in the aquatic environment as a result of their use as biocides in marine paints. Among them, tri-n-butyltin (TBT) derivatives are currently used the most, even though their toxicity to aquatic organisms may be extremely high.[1–5] The effects on the environment depend on TBT concentration and its persistence in the medium, as well as the toxicity of the products of degradation. These products include dibutyltin (DBT), monobutyltin (MBT), and also monomethyltin (MMT), dimethyltin (DMT) and trimethyltin (TMT) resulting from the methylation of released tin(IV). Several studies have shown the occurrence of these compounds in sea and lagoon waters,[6] in sediments[7–9] and in aquatic organisms.[10–12] The origin of TBT degradation or tin methylation may be either biotic or abiotic.

A number of authors have demonstrated the ability of various microorganisms isolated from seawater or sediments to transform organotin compounds, tin(II) or tin(IV).[13,14] In a previous paper, we have studied the bioconversion of TBT, DBT and MBT compounds in the presence of pure strains of growing microorganisms. In appropriate culture media and under well-defined conditions, it was shown that biotic degradation of tributyltin chloride far exceeded abiotic decomposition. In most cases, monobutyltin was formed in higher yields than the other metabolites, and all microorganisms were able to methylate tin(IV) to trimethyltin.[13]

In the present work, the conditions of incubation of the microorganisms were modified in the hope of further increasing the yield of TBT degradation and also possibly to select a microorganism to study the phenomenon at the enzymic level. Contrary to organomercurials,[15] the mechanism of bacterial or fungal resistance to organostannanes (especially TBT) has not been elucidated, although it could be very important in relation to environmental detoxification. The use of resting cells with the biomass isolated from the culture medium and resuspended in buffer was expected to improve the yields of bioconversion and to avoid the possible occurrence of an abiotic degradation of organotins due to the partially metabolized culture media. The production of methyltin derivatives from inorganic tin(IV) under the same conditions has also been examined.

MATERIALS AND METHODS

Organotin compounds were purchased from Merck (Darmstadt, Germany), inorganic salts from Prolabo (Paris, France), and culture

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medium nutrients from Fluka (Buchs, Switzerland).

Seven microorganisms from our collection were selected for their ability to grow in the presence of controlled amounts of organotin compounds, namely: *Aspergillus tamarii* (NRRL 427), *Chaetonimium globosum* (NRRL 1870), *Penicillium citrinum* (NRRL 1843), *Pseudomonas fluorescens* (NRRL B-10), *Saccharomyces cerevisiae* (NRRL Y-129), *Saccharomyces cerevisiae* (NRRL Y-409) and *Schizosaccharomyces pombe* (NRRL Y-9). Microorganisms were obtained from Northern Regional Laboratories, Agricultural Research Services, Peoria, IL, USA.

The maximal level of initial TBTCl concentration compatible with microorganism growth was adjusted to 10 $\mu g\,l^{-1}$ in agreement with the conclusions of our previous study.[13] The biomass for resting-cell experiments was obtained according to a two-stage procedure.[13,16] A sample of microorganism culture on agar slant was used to inoculate 50 ml of the appropriate medium in a 250-ml culture flask. After 48 h at 28 °C in a longitudinal shaker (120 rpm), under aerobic conditions, all the suspension was used to inoculate 400 ml of fresh medium in a 2-litre flask. After two to four days, depending on the microorganism, the biomass was filtered (fungi) or separated from the liquid phase by centrifugation (yeasts and bacteria). The biomass was washed three times with sterile phosphate buffer (50 mmol l^{-1}, pH 6.8) and resuspended in 50 ml of fresh phosphate buffer in a 250-ml flask. After 8 h at 28 °C, the organotin compound dissolved in methanol (50 µl of a 10 mg l^{-1} solution: initial concentration 10 g l^{-1}) or $SnCl_4$ dissolved in concentrated sulfuric acid (50 µl of a 20 mg l^{-1} solution; initial concentration 20 $\mu g\,l^{-1}$) was added to the suspension. The medium was then shaken at 28 °C for five days (120 rpm) and aliquots were analyzed using a procedure already reported in a previous publication.[13] Organotin compounds were separated from the biomass by stirring for 12 h in pure acetic acid and centrifugation.[17] The quantitative analysis successively involved hydride generation, cryogenic trapping, gas chromatography and atomic absorption spectroscopy.[17–19] Reproducibility of the method was 6%, with lower limits of detection from 0.2 to 0.3 ng l^{-1} for methyltins and monobutyltin, 0.4 ng l^{-1} for dibutyltin, and 0.6 ng l^{-1} for tributyltin.[18] Standard regression curves were used for the titrations. This procedure is not valid for inorganic precise titrations of tin(IV) since sodium borohydride used for hydride generation contains traces of this cation.

Volatile tin compounds formed after incubation of TBT with *P. citrinum* for 84 h were conveyed by a sterile air flow (100 ml min^{-1}) into a trap containing pure acetic acid and glass bearings at 28 °C. When using this kind of trap, it was necessary to introduce sodium borohydride to determine the type of tin compounds trapped, as organostannanes are not stable in acetic acid. Therefore another trap was used in a 10-h experiment, the microcolumn trapping the reaction products (Chromosorb WPH 80–100 mesh, OV 101 10%) being immersed in ethanol–liquid nitrogen (temperature about −47 °C). These methods did not trap the totality of the volatile tin compounds, and the fraction of these compounds was estimated from the difference between the initial tin introduced and the total tin recovered.

The experimental results are expressed as the average of three measurements. In all cases, the standard deviation was less than 10%.

RESULTS AND DISCUSSION

Although the biotransformation of alkyltins in the environment is a well-established phenomenon, the actual enzymic processes responsible for these degradations have not been determined.[20] Two enzymically catalyzed processes have been widely observed: methylation of inorganic tin(II) or tin(IV) and dealkylation of mono- or poly-alkyl tin derivatives (especially TBT).[20] Methylcobalamin derivatives have been identified as the probable methyl source in some methylation reactions,[21] and hydroxybutyltin compounds have been shown to be intermediates in the microsomal monooxygenase metabolism of TBT.[22] Prior to studying the metabolism of alkyltins by microorganisms, it seems necessary to select a system efficient in dealkylation or methylation reactions. In a previous study, we found a number of microorganisms inducing debutylation of TBTCl and methylation of released inorganic tin(IV) in culture medium. Using the corresponding resting cells in inorganic buffer instead of growing cells in nutrients was expected to improve the yield of the enzymic debutylation of TBTCl or tin methylation with the organotin substrate as the sole carbon source in the reaction medium. Such experiments involving TBT and resting cells of microorganisms isolated from the

Table 1 Biotransformation of tributyltin chloride in resting-cell conditions: recovery of substrate and products (% of Sn)

Microorganism	Recovery						Volatile tin products	Biodegradation (%)[b]
	TBT		DBT		MBT			
	A[a]	B[a]	A[a]	B[a]	A[a]	B[a]		
C. globosum	58	1	1	<1	2	7	31	39
P. citrinum	21	0	3	0	9	7	60	77
A. tamarii	84	2	4	<1	2	2	0	12
S. pombe	73	0	4	<1	1	3	19	25
S. cerevisiae	84	0	2	0	1	5	0	14
S. cerevisiae	88	0	3	0	2	10	0	10
P. fluorescens	61	0	3	0	1	22	13	37

[a] A, biomass; B, solution. [b] Lower limit of biodegradation taking into account the nonenzymic transformation of TBT (2%) in buffer.

environment or from pure strains have seldom been successful in the past, presumably due to the excessive concentration of substrate.[14]

The results of TBTCl biotic degradation in the presence of resting cells of the microorganisms listed in the Materials and methods section are presented in Table 1. Abiotic degradation of TBTCl in phosphate buffer without microorganism was very low (2%) and yielded DBT and MBT. Biotic degradation was expressed as the difference between the observed total TBT degradation and the abiotic degradation. Comparing the data of Table 1 with the results of experiments with the same growing microorganisms[13] leads to two important conclusions: (1) in most cases, the yield of TBT bioconversion with resting cells was significantly increased; (2) in half the experiments, a large proportion of metabolites were volatile tin compounds. In solution, the products were identified as MBT and DBT, along with unreacted TBT. As with growing cells, MBT was the major product and was recovered mostly from the liquid phase. In no case were inorganic tin or methyltin compounds detected, either in the liquid phase or associated with the biomass. With *S. pombe*, a small amount of an unknown product was found in solution: its retention time, which is intermediate between those of DBT and TBT, suggests a mixed methyl butyl tin structure for this compound.

Four microorganisms out of seven volatilized substantial amounts of TBTCl: *P. fluorescens, S. pombe, C. globosum* and *P. citrinum*. Analysis of the volatile fraction was attempted in the case of *P. citrinum*, which yielded the highest proportion of these products. Using acetic acid to trap the volatile products resulted in the detection of MBT and inorganic tin(IV) as major products and MMT, DMT, TMT, DBT and TBT as traces, after the usual treatment with sodium borohydride. The amount of tin compounds trapped in acetic acid was markedly lower than the amount of volatile products formed. These experiments did not indicate the nature of the volatile compounds, due to their probable decomposition in the acidic medium and the treatment with $NaBH_4$. However, direct analysis of the products trapped on a column immersed in ethanol–liquid nitrogen and without treatment by sodium borohydride showed the presence of tributylstannane. This suggests that the volatile products were, at least in part, stannanes derived from TBTCl or its metabolites.

The bioconversion of dibutyltin chloride ($DBTCl_2$) under similar conditions and with the same set of microorganisms yielded the values reported in Table 2, with abiotic degradation taken into account. The only detected metabolite in the liquid medium was MBT, its proportion varying from 13 to 45% of the initial tin, depending on the microorganism. Moreover, all microorganisms volatilized large amounts of organotin compounds (20–56%). Taken together, the results show that $DBTCl_2$ is very efficiently bioconverted in the presence of resting cells in phosphate buffer, since overall yields of $DBTCl_2$ transformation varied from 35 to 98% (Table 2). Similarly, MBT is transformed in good yields, ranging from 52 to 74%, but only volatile tin compounds were produced in this case, and no evidence of the presence of inorganic tin(IV) in the medium was found (Table 3).

Table 2 Biotransformation of dibutyltin chloride in resting-cell conditions: recovery of substrate and products (% of Sn)

	Recovery					
	DBT		MBT			
Microorganism	A[a]	B[a]	A[a]	B[a]	Volatile tin products	Biodegradation (%)[b]
C. globosum	1	0	15	30	54	98
P. citrinum	5	0	18	25	52	94
A. tamarii	44	3	10	11	32	52
S. pombe	57	7	5	11	20	35
S. cerevisiae	25	0	7	12	56	74
S. cerevisiae	44	6	5	9	36	55
P. fluorescens	55	9	5	8	28	35

[a] A, biomass; B, solution. [b] Taking into account the nonenzymic transformation of DBT (1%) in buffer.

Finally, under the same conditions, there was no conversion of $SnCl_4$ into organotin compounds, either soluble or volatile. This contrasts with the results of similar experiments involving the same microorganisms actively growing in the presence of nutrients in which $SnCl_4$ was transformed to soluble methyltin compounds.[13]

The results of the bioconversions of butyltin compounds in the presence of microorganisms in the resting state confirm the conclusions of our previous study with growing microorganisms.[13] In all cases, the major soluble metabolite obtained in the degradation of TBTCl or $DBTCl_2$ was MBT as an ionic species in association with an anion (Cl^-, HO^-).[14] On the whole, the degradation process giving soluble products appears more efficient with resting cells than with with growing cells. However, the most significant result obtained with resting cells was the conversion of butyltin chlorides to volatile products, presumably hydrides of butyltin compounds. Therefore, three different types of reaction seem to occur depending on the conditions: (1) degradation of butyltin chloride to lower homologues; (2) methylation of inorganic tin(IV); (3) reduction of alkyltin chlorides to insoluble hydrides.

Table 3 Biotransformation of monobutyltin chloride in resting-cell conditions: recovery of substrate and products (% of Sn)

	Recovery			
	MBT			
Microorganism	A[a]	B[a]	Volatile tin products	Biodegradation (%)[b]
C. globosum	10	17	73	67
P. citrinum	15	4	81	74
A. tamarii	9	20	71	64
S. pombe	18	23	59	52
S. cerevisiae	4	30	66	59
S. cerevisiae	5	32	63	56
P. fluorescens	4	36	60	53

[a] A, biomass; B, solution. [b] Taking into account the nonenzymic transformation of MBT (7%) in buffer.

In contrast with the experiments with growing cells, only traces of methyltin compounds were produced with resting cells, presumably due to the absence of nutrients in the medium as a source of necessary cofactors. Based on recent literature data, two possible enzymic mechanisms may be suggested for debutylation of TBT derivatives: either via hydroxylation of the alkyl groups or via protonolysis of carbon–tin bonds catalyzed by a lyase. Hepatic monooxygenases have been shown to hydroxylate tributyltin acetate particularly at the α- or β-position of the alkyl groups, thus inducing the cleavage of carbon–tin bonds.[22] However, hydroxylated metabolites of alkyltin compounds have never been identified in bacterial or fungal decompositions of these compounds, and the generality of this mechanism remains to be established. Bacterial resistance to organomercurials involves an organomercurial lyase which efficiently catalyzes the protonolysis of alkyl–mercury bonds to hydrocarbons (R–H) and inorganic mercury(II). A mercuric reductase subsequently effects the reduction of mercury(II) to mercury(0),which evaporates from the cell.[23] Organomercurial lyase has been shown to be much less efficient in catalyzing the dealkylation of alkyltins, especially TBT oxide, which is not a

substrate for the lyase.[15] Therefore, the existence of an organotin lyase has to be demonstrated before taking into account this type of dealkylation process, and no specific protonolytic enzyme involved in organotin compounds has yet been isolated. Although dimethyl- and trimethylstannanes have been observed in microbial methylations of inorganic tin cations, no mention of butylstannanes in the bioconversion of TBT derivatives has been made in the literature.[14] Our finding that a fairly large proportion of TBT was metabolized to volatile compounds by microbial resting cells and that tributylstannane was detected among the metabolites from one selected microorganism imply the occurrence of a reductive cleavage of the carbon–tin bond to a stannane and probably a hydrocarbon.

CONCLUSIONS

Our results confirm the conclusions of an earlier study of TBTCl microbial metabolization by the same set of pure strains in culture medium.[13] Although the yields of enzymic degradation were greater when the experiments were conducted with butyltin derivatives as the sole carbon source, with all microorganisms in culture medium, as well as with resting cells, the major products were monobutyltin derivatives. The results of the present study also suggest that detoxification may occur through volatilization, probably to butylstannanes. The enzymes responsible for these processes remain to be identified and their mechanisms clarified.

REFERENCES

1. R. J. Maguire, *Appl. Organomet. Chem.* **1** 475 (1987).
2. M. Rexrode, in: *Ocean '86, Proc. Int. Organotin Symp. Washington DC*, **4**, 1443 (1986).
3. R. J. Maguire, in: *Ocean '86, Proc. Int. Organotin Symp, Washington DC*, **4**, 1252 (1986).
4. C. Alzieu and Y. Thibaud, *Bull. Acad. Nat. Med.* **167**, 473 (1985).
5. P. E. Gibbs, P. L. Pascoe and G. R. Burt, *J. Mar. Biol. Ass. UK* **68**, 715 (1988).
6. R. Pinel, M. Astruc and O. Donard, *L'Actual. Chim.*, **110**, (1990).
7. F. E. Brinckman, J. A. Jackson, W. R. Blair, G. J. Olson, and W. P. Iverson. in: *Trace Metal in Sea Water*, Wong, C. S., Boyle, E., Bruland, K. W., Burton, J. D. and Goldberg, E. D. (eds), NATO Conference Series IV, Plenum Press, New York, 1982 pp. 39–72.
8. Y. K. Chau, P. T. S. Wong, O. Kramar and G. A. Benger, in: *Methylation of Tin in the Aquatic Environment*, Canada Center for Inland Waters, Burlington, Ontario, Canada, 1981.
9. C. Barguigiani, T. Ristori and R. Bauleo, *Environ. Technol.* **12**, 1175 (1991).
10. O. Donard, F. T. Short and J. H. Weber, *Can. J. Fish Aquat. Sci.* **44**, 140 (1987).
11. M. J. Waldock, J. E. Thain and D. Miller, in: *The Accumulation and Depuration of Bis(tributyltin) Oxide in Oyster: A Comparison between the Pacific Oyster* C. gigas *and the European Flat Oyster* O. edulis, International Council for the Exploration of Sea, CM1985, E:52, 1985.
12. R. J. Maguire, J. H. Carey and E. J. Hale, *J. Agric. Food Chem.* **31**, 1060 (1983).
13. O. Errecalde, C. Astruc, G. Maury and R. Pinel, *Appl. Organomet Chem.* **9**, 23 (1995).
14. J. J. Cooney, *J. Ind. Microbiol.* **3** 195 (1988).
15. A. E. Walts and C. T. Walsh, *J. Am. Chem. Soc.* **110**, 1950 (1988).
16. D. Perlman, in: *Applications of Biochemical Systems in Organic Chemistry*, Jones, J. B., Sih, C. J. and Perlman, D. (eds), Techniques of Chemistry Vol. 10, John Wiley, Chichester, 1976, p. 47.
17. R. Lavigne, R. Pinel and M. Astruc, *Environ. Pollut* **57**, 149 (1989).
18. V. Desauziers, F. Leguille, R. Lavigne, M. Astruc and R. Pinel, *Appl. Organomet. Chem.*, **3**, 469 (1989).
19. F. Leguille, A. Castetbon, M. Astruc and R. Pinel, *Environ. Technol* **14**, 949 (1993).
20. D. Barug, *Chemosphere* **10**, 1145 (1981).
21. L. J. Dizikes, W. P. Ridley and J. M. Wood, *J. Am. Chem. Soc.* **100**, 1010 (1978).
22. E. C. Kimmel, R. H. Fish, and J. E. Cassida, *J. Agric. Food Chem.* **25**, 1 (1977).
23. M. J. Moore, M. D. Distefano, L. D. Zydowsky, R. T. Cummings and C. T. Walsh, *Acc. Chem. Res.* **23**, 301 (1990).