Bull. Environ. Contam. Toxicol. (1992) 48:599–607

Environmental Contamination and Toxicology

Biosorption and Degradation of Butyltin Compounds by the Marine Diatom *Skeletonema costatum* and the Associated Bacterial Community at Low Temperature

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Since the discovery of their biocidal properties in the 1950s, organotin compounds have found a large spectrum of industrial applications such as wood and textile preservatives, fungicides and pesticides, and anti-fouling paint on ships and fishing equipment. The fate and environmental impact of butyltins have been the subjects of a large body of research in the last decade (Clark et al 1988 and references therein). Butyltins are toxic to aquatic microorganisms; they inhibit the growth rate of many species of phytoplankton (Walsh et al. 1985) and reduce primary productivity of natural communities of freshwater and marine algae (Wong et al. 1982). The sensitivity of aquatic algae to butyltins shows a large disparity which could be associated with the ability of some species to incorporate and sequester tin compounds in cell tissues and/or degrade tributyltin into less toxic di- and mono-butyltins (Maguire et al. 1984).

The toxicity of tributyltin oxide to the marine diatom *Skeletonema costatum* was studied by many authors (Thain 1983; His et al. 1986; Walsh et al. 1985; Beaumont and Newman 1986) who observed an inhibition of growth rate at TBT test concentrations below 0.5 μg/l and LC_{50}s ranging from 5 to 18 μg/l for a series of TBT halides and oxides. According to Lee et al. (1989), *S. costatum* was particularly efficient to degrading TBT in dibutyltin and hydroxylated by-products.

The objective of this work was to evaluate the potential role of the diatom *S. costatum* and the associated bacterial community in the food transfer of butyltin compounds to grazing and filter-feeding marine organisms by measuring the surface cell adsorption, incorporation and

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degradation by cells. The influence of the bacterial community was also examined. The study reports the fate of a sublethal concentration (1 μg/l) of individual tri-, di- and mono-butyltin chlorides in the presence and absence of *S. costatum* over a 96 h time period at a temperature of 4 °C.

MATERIALS AND METHODS

Algae, obtained from the culture collections of the Institut National de la Recherche Scientifique, were cultured in 0.2 μm filtered seawater (salinity 30.5 °/$_{oo}$) supplemented with nutrients (phosphate, nitrate and silicate salts), oligoelements (iron) and vitamins (thiamine and cyanocobalamine). Algae were transferred from batch culture (a 50 L transparent plastic bag with a continuous air bubbling) to sterile 4 L glass bottles seven days after the inoculation; the culture was near the end of the exponential growth phase. Tri-, di- and mono-butyltin chlorides were respectively introduced at a test concentration of 1 μg/l in three different bottles and one flask was kept as the control. Algae were exposed to the natural light cycle in a greenhouse and the temperature was maintained at 4 °C to simulate spring conditions prevailing in the Saint Lawrence Estuary, the light period of which was about 14 h. Two additional bottles were filled with sterile seawater and 0.7 μm filtrated seawater, respectively, and spiked with a test mixture of tri-, di- and mono-butyltin chlorides to a total concentration of 2 μg/l and exposed to natural light with algal cultures. After 96 h, the culture media were centrifuged at 5,000 g for 15 min and supernatant seawater media samples were stored in brown bottles at -20 °C. Cells were washed twice by centrifuge with 40 ml acidic seawater (HCl pH 5) and the washing solutions and cells were preserved for organotin analysis.

Butyltin compounds were extracted and analyzed following a method described elsewhere (Reader 1991). Briefly, organotins were extracted from the seawater using tropolone (0.2%) in dichloro-methane, derivatized in magnesiumhexyl bromide solution (2 M in ethyl ether) and quantified by gas chromatography/ion trap spectroscopy (GC/ITD). Phytoplankton cells were freeze-dried and pulverized prior to the extraction procedure. The detection limit of the GC/ITD system was estimated to be 5 ng/l assuming 100 % recovery of butyltins. The coefficient of variation (CV) for the whole

method (extraction, derivatization, clean-up and separation by GC) was $\leq$ 10%.

Viable heterotrophic bacteria were estimated using the Most Probable Number (MPN) procedure with the 2216E marine broth Difco. Visual growth after incubation (18°C for 20 days) was the criterion for scoring the MPN plates (3 plates per dilution). Samples were collected at 0,72 and 96 h (Table 1) and counted for bacteria before centrifuging.

RESULTS AND DISCUSSION

Viable bacteria counts observed in culture media and filtered seawater (SW) are reported in Table 1. Seawater filtered through a 0.2 μm glass filter was sterile whereas the bacterial community grew slowly in the sample filtered through a 0.7 μm glass filter .

Table 1. Viable bacteria counts observed during the 96 h experimental period .

Elapsed time (h)	Filtered SW (0.2 μm)	Filtered SW (0.7 μm)	All culture media
0	0	500	$\geq$10 000
72	0	2350	$\geq$10 000
96	0	2680	$\geq$10 000

Bacterial counts were high ($\geq 10^4$ cells/ml) in all culture containers because the algal strain was not axenic and the rich medium of the batch culture enhanced the growth rate of the bacterial community associated with diatom cells.

The cell densities of *S. costatum* were monitored in bottles contaminated with butyltins and in a control (Fig 1). Beaumont and Newman (1986) have reported a high sensitivity of *S. costatum* to tributyltin oxide (TBTO); cells died within 2 days when exposed to

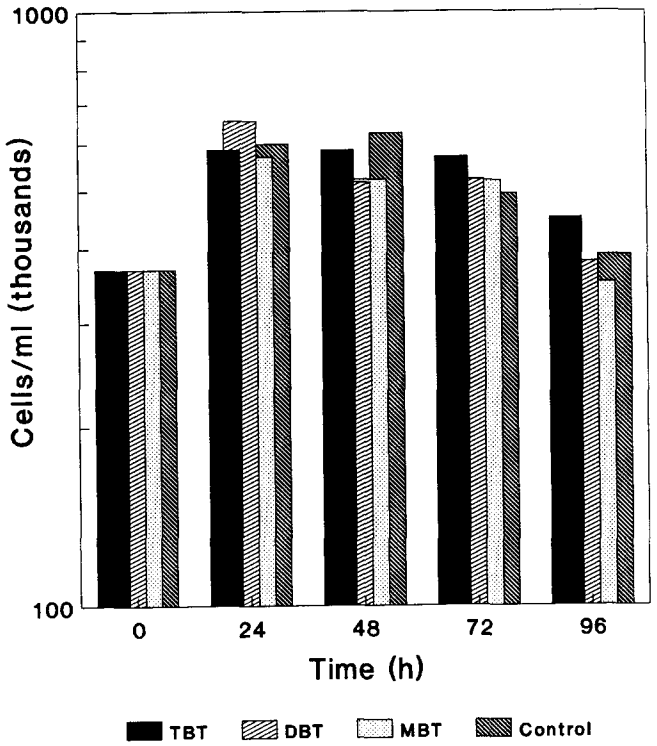

Figure 1. Distribution of diatoms *Skeletonema costatum* in control and culture media subjected to tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) at 1 μg/l.

TBTO at 5.0 μg/l. Walsh et al. (1985) observed growth rate inhibition of *S. costatum* (in an axenic batch culture at ambient temperature) in the presence of TBT acetate, TBTO and TBT halides at 0.36, 0.33 and between 0.25 and 0.50 μg/l, respectively.

In the present study, the cell densities of *S. costatum* were not affected significantly in the presence of tri-, di- and mono-butyltin chloride at 1 μg/l for a period of 96 h. All cell counts increased in the first 24 h of the exposition period, reached a 24 h plateau and then declined in the last 48 h of the experiment. The rate of decline (about 60,000 cells/day) was the same for all cultures and corresponds to the normal decay of these batch cultures after they reached a maximum cell density.

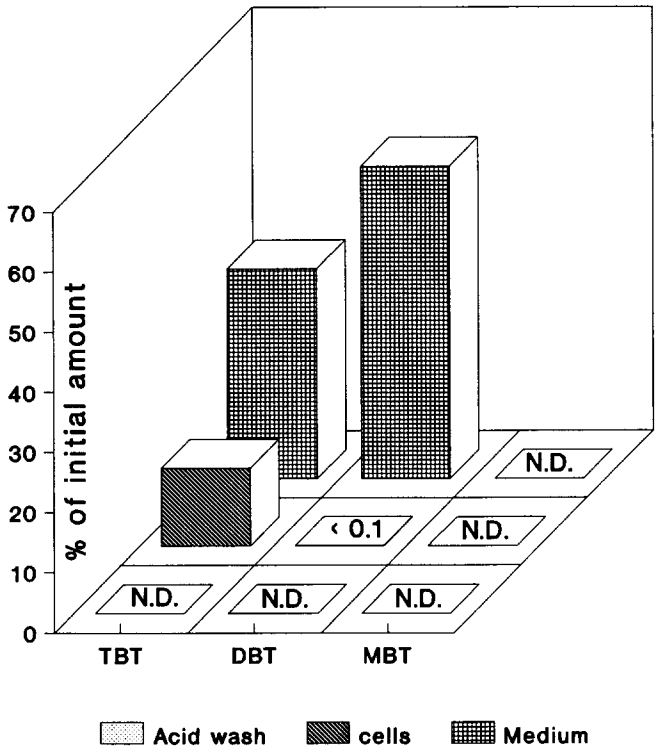

Figure 2. Distribution of butyltin species recovered from *S. costatum* cultures after a 96 h contact period with TBT at 4 °C. Values are expressed in % of the initial amount of tributyltin introduced in the culture medium (1 µg/l). N.D. means butyltin compounds were not detected at a detection limit of 5 ng/l.

Some environmental factors, such as the contamination with butyltins near the end of the logarithmic phase of growth, a low incubation temperature (4 °C) and the presence of an important bacterial community, could have had a significant role in the apparent resistance of these cells to the imposed toxic stress when compared to the low tolerance level reported by Walsh et al. (1985).
The results of biosorption and degradation of tributyltin by *S. costatum* are summarized in Figure 2. The most striking feature is the recovery of more than 50% of the initial amount of TBT (1 µg/l) as dibutyltin in the culture medium after centrifugation of cells. The rest

of TBT was left intact in the culture medium (35%) or incorporated in cells (15%). In a comparable experiment, Lee et al. (1989) also recovered 50% of the initial TBT as DBT but only 25% remained unchanged TBT in the culture and the last 25% was metabolized into hydroxylated by-products. An attempt to detect by GC-ITD some hydrobutyltin compounds similar to those reported by Lee et al. (1989) was unsuccessful at the detection limit of 5 ng/l. Only traces of DBT were detected in cells. No butyltin species were recovered in acidified seawater (pH 5) used to wash cells. This indicates that butyltins were not physically adsorbed on the external surface of cells but were incorporated into the cell tissues or, alternatively, were strongly bounded to surface sites with chemical bonds resistant to acid hydrolysis.

In the culture sample contaminated with DBT only, the contaminant was quantitatively recovered in the medium, unchanged, and was not detected in cells nor in the acid wash solution. Similarly, no butyltin was recovered in cells extracted from the sample contaminated with MBT. Finally, 80% of the initial TBT was recovered from the bottle containing 0.7μm filtered seawater.

The high recovery of TBT from the filtered SW, exposed to sunlight and containing only bacteria (Table 1), confirmed that direct photolysis and bacteria alone did not significantly contribute to TBT degradation (Maguire et al. 1983; Lee et al. 1989). The most common mechanism of bacterial resistance to the inhibitory effects of organometals involves cleavage of the metal-carbon bond (Thayer 1984). Even if a significant microbial action on butyltin compounds was reported for many strains, degradation rate constants were slow and half-lives exceeded two weeks for TBTO and TBTCl under optimal laboratory conditions for bacterial growth (Zuckerman et al 1978). Bacterial degradation cannot be totally ruled out in experiments reported here, but the short exposure period (4 days), the low temperature of cultures (4 °C) and the strong competition from algae should have reduced to minimum the contribution of bacteria to the degradation process.

We found that *S. costatum* and the associated bacterial community were not active on partly dealkylated butyltins as DBT and MBT were quantitatively recovered from culture media in absence of other

degradation products. The important incorporation of TBT within *S. costatum* cells has not been reported previously but the accumulation of TBT by phytoplankton has been observed by Maguire et al. (1984) for a freshwater green alga, *Ankistrodesmus falcatus*, exposed to high concentrations of TBT (20-40 μg/l) for four weeks. The presence of 15 % TBT and traces of DBT inside cells and the absence of acid-leachable butyltins at the surface of the cells support the hypothesis that degradation takes place in the cytosol and not on the external surface of the diatom as opposed to surface photo-oxidation or a microbial degradation at the surface of cells. The procedure for the separation of diatoms from the culture medium by low energy centrifugation (5,000 g) did not sediment down bacteria free in the solution. Butyltins potentially bioaccumulated in bacteria, if any, were accounted for in the culture medium (Fig. 2). Bacteria attached to the surface of cells were more probably washed out by the acid procedure (HCl solution at pH 5) and associated butyltins, if any, were recovered in the acid-wash fraction. As very few butyltins were recovered in acidic fractions, it arrears to be an indication that bacteria attached to cells did not bioaccumulate TBT or DBT at a detectable level.

Results presented here support previous results on the particular capability of *S. costatum* to degrade tributyltin chloride, even at 4 °C, without lethal toxic effects and without clear evidence of a contribution from the associated bacterial community. The rapid degradation of TBT into DBT by *S. costatum* at low temperature is an important benefit in the natural depuration process, but the bioaccumulation of the toxic compound by cells (15% of the initial amount) is a major drawback since the process will contribute to the incorporation of TBT into the food chain and make it available to zooplankton and planktonic larvae which are strongly affected by TBT both from the medium and the food (Beaumont and Budd 1984; Bryan et al. 1986; Laughlin et al. 1989).

Acknowledments. This work was supported by NSERC Canada and FCAR Québec. This publication is a contribution of the Oceanographic Centre of Rimouski - a partnership of INRS (Institut National de la Recherche Scientifique) and UQAR (Université du Québec à Rimouski) operating under the auspices of the University of Québec .

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Received June 3, 1991; accepted December 1, 1991.