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Biosorption and Biodegradation of the Antifouling Compound Tributyltin (TBT) by Microalgae

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Abstract: The biosorption and biodegradation of tributyltin (TBT) at its sublethal concentration of the two unicellular algae *Nannochloropsis oculata* (a wall-celled stigmatophyte alga) and *Dunaliella parva* (a wall-less chlorophyte alga) are investigated. The initial concentration of TBT in culture media of *N. oculata* was reduced by 46% within two days, while in *D. parva* it decreased stepwise reaching 50% within 6 days. At the end of the experiment (12 days) both algae sorped nearly 80% of the initial TBT content. The extracellular analysis of TBT cleared that biosorption by the algal cell wall of *N. oculata* was the major mechanism in reducing 40% of the initial TBT in the first two days (6% may be diffused through the cell wall of the alga). On the contrary, a very small amount of TBT was recorded on the outer membrane of *D. parva*, which disappeared after 4 days. The occurrence of DBT and MBT during the first few days in the culture media of *D. parva* and their absence in culture of *N. oculata* may support the fact that *D. parva* has a special internal mechanism for degrading TBT to the less toxic fractions DBT and MBT. In both algae the diffusion of TBT - which started early in *D. parva* – caused gradual increase in the intracellular concentration of TBT as time proceeded reaching maximum after 6 days in *D. parva* and 8 days in *N. oculata.* The gradual increase in the content of MBT on the expense of TBT and DBT may be the reason for the tolerance ability of *D. parva* to toxicity of TBT.

Key words: Antifouling · Biosorption · Biodegradation · Organotin · Microalgae

INTRODUCTION

Organotin compounds are universally used as effective antifouling chemicals [1]. Antifouling coatings are the most reliable way to prevent biofouling of immersed surfaces [2]. Commercial antifouling formulation containing TBT are the major source of organotin contamination in coastal waters [3]. The biofouling slimes that form on ship hulls are reported to raise fuel consumption by as much as 30% in some cases [4]. Ship hulls is a powerful way of spreading species to new parts of the world oceans leading to bioinvasion, which is now recognized as a major treat to biodiversity [5].

The widespread use of TBT as an ingredient of antifouling paints preservative and biocides has led to serious environmental contamination [6, 7]. This opened a subject of research work to study the toxic effect of TBT on aquatic animals, fish and microorganisms including algae. Many authors reported that abiotic degradation of TBT such as photolysis, chemical and thermal cleavage had negligible effect in the natural breakdown of TBT [7-9]. However, much of the evidence suggests that biotic degradation by microorganisms such as bacteria, fungi and microalgae is the major pathway for removal of TBT in the aquatic environment [10, 11]. The tin atom in a form of polymeric salt can be exchanged with sodium ions in seawater. As the ion exchange reaction proceeds the polymers become soluble and leach out by flow of seawater [12]. Although it has demonstrated that microalgae, fungi and bacteria are able to biosorb and debutylate TBT into less toxic DBT, MBT and inorganic tin [13, 14] information is still severely limited [15]. The removal efficiency and degradation ability seemed vary from species to species [7, 10, 13, 14]. The tolerance of an organism will, therefore, be related to its ability in degrading TBT to less toxic species.

The present study aimed to investigate the ability of the wall less green microalga *Dunaliella parva* and the stigmatophyte microalga *Nannochloropsis oculata* in biosorption and biodegradation of the organotin antifouling compound tributyltin (TBT).

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MATERIALS AND METHODS

Biological Materials: The biological materials chosen for this work were the golden unicellular stigmatophycean alga *Nannochloropsis oculata* purchased from the Solar Energy Research Institute (SERI) culture collection in Golden, Colorado, USA and the wall less unicellular flagellated alga *Dunaliella parva* obtained from the UTEX – the culture collection of algae, at the University of Texas at Austin.

Chemicals: The chloride salts of TBT, DBT and MBT and tripentyltin were purchased from Merk, Schuchardt, Germany. Most of the other chemicals used were from Sigma chemical Co. (USA). Stock solutions of 10 mg/l of the standards TBT, DBT, MBT and tripentyltin (internal standard) were prepared in acetone and stored in dark at 4°C. Tripentyltin was used as internal standard to adjust the loss of butyltin compounds during the extraction and injection process.

The spiking of TBT in acetone in the present study was controlled so that the final concentration of acetone added was below 1.67% (v/v) in the medium. Lee (1997) and Tsang *et al.* (1999) [7 and 16] found that the toxicity of acetone on algal cells at concentration up to 1.76(v/v) posed no significant effect on population growth of algal species.

Culturing: The axenic algal materials were grown in enriched sea water medium as described by Boussiba *et al.* [17]. Sterilization was carried out by autoclaving the flasks containing the media at 120°C for 20 min. The two selected algae were cultured each in Erlenmeyer pyrex-glass flasks (capacity 500 ml) each contained 300 ml culture medium.

A known number of cells at logarithmic phase of growth were used as inocula for experimental studies (3.0 x 10^6 cells/ml). The cultures were grown under controlled laboratory conditions (temperature at 28°C±3°C and light at 80μmol $m^{-2}s^{-1}$ with a 16h light: 8h dark cycle). Preliminary experiments showed that after 8 days, the two organisms reached their late logarithmic phase of growth and the sub lethal concentration of TBT was predetermined as 75 μg/l and 40 μg/l for *N. oculata* and *D. parva* respectively. So after 6 days of growth these two concentrations of TBT were added to the culture flasks. At regular intervals (couple of days), two tested flasks were subjected for analyses. According to the method described by St-Louis *et al.* [18], the determination of butyltin species remained in the medium and associated with the algal cells (whether extra- or intra-cellular) were conducted. So from each culture flask 100 ml was centrifuged and the dry weight of the cell pellet was determined by drying at 105°C until constant weight was attained and kept in a desiccator. The various butyltin species and their concentrations were analyzed in the supernatant of the remains volume of cultures (200 ml) after centrifugation. Extraction of butyltin species in the supernatant was conducted according to Muller [19] and Waldock *et al.* [20]. The collected cell pellet obtained after centrifugation was washed with deionized water and resuspended in dilute HCl (pH 3.0) to solubilize the butyltin species being adsorped on the outer cell surface, then centrifuged. The cell pellet collected after the acid desorption process was homogenized in liquid nitrogen to determine the intracellular butyltin species.

Analysis: The TBT concentrations and its degradative fractions in water culture and extracts were analyzed at definite days. The samples were acidified by addition of known volume of concentrated HCl and stored in dark glass bottles at 6-8°C until analysis. The samples were neutralized with NaOH and an internal standard of tripentyltin was added before extraction with dichloromethane in a separation funnel gently shaken for 15 min. The organic phase was then blown down to a volume by a gentle stream of dry air.

The separation and detection of organotin species was performed by gas chromatography with a flame photometric detector (GC-FPD, Hewlett-Packard 5890) by using a special temperature program according to Waldock *et al.* [20]. Members of the organotin family were identified on the basis of their retention times and quantified by peak area integration reference to external standards.

RESULTS

The results obtained for biosorption and degradation of TBT in the medium of *Nannochloropsis oculata* were recorded in Fig. 1. From these results, it is clear that during the first 2 days, *Nannochloropsis* biosorped about 33 μg/l (i.e. 45%) from the initial 70 μg/l of TBT. At the end of the experiment (12 days) the level of TBT in the medium decreased to 10 μg/l i.e. 94.4% reduction in the initial amount of TBT. However, traces of DBT were detected after two days while MBT appeared at the 4th day of culturing. Both slowly and gradually increased reaching maximum at the end of the experiment (8 and 5 μg/l for DBT and MBT, respectively).

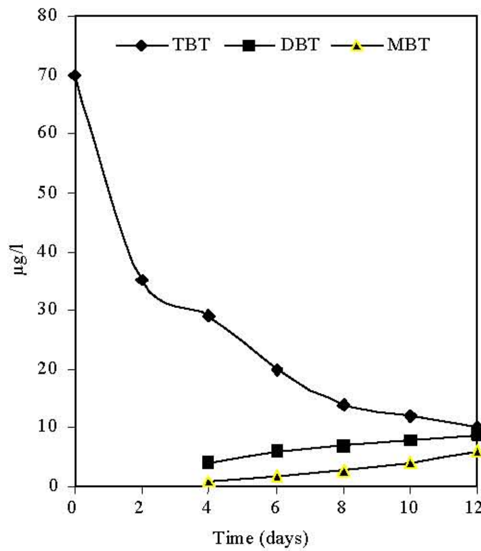

Fig. 1: The content of TBT, DBT and MBT in the culture medium containing the sublethal concentration of TBT for *Nannochloropsis oculata*

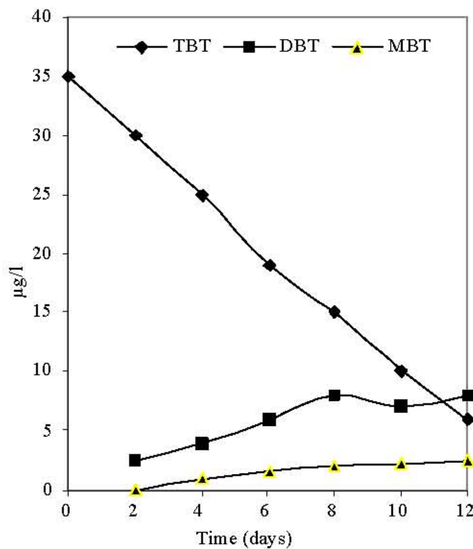

Fig. 2: The content of TBT, DBT and MBT in the culture medium containing the sublethal concentration of TBT for *Dunaliella parva*

The degradation capacity of TBT by *Dunaliella parva* (Fig. 2) differed greatly from that of *Nannochloropsis*. After 6 days the concentration of TBT in the culture medium of *D. parva* decreased to 19 µg/l i.e. 45.5% of total TBT in the culture. However, at the end of the experiment (12 days) the final concentration of TBT in the medium reached 6 µg/l i.e. nearly 85.3% of total TBT in the culture. On the contrary, DBT was

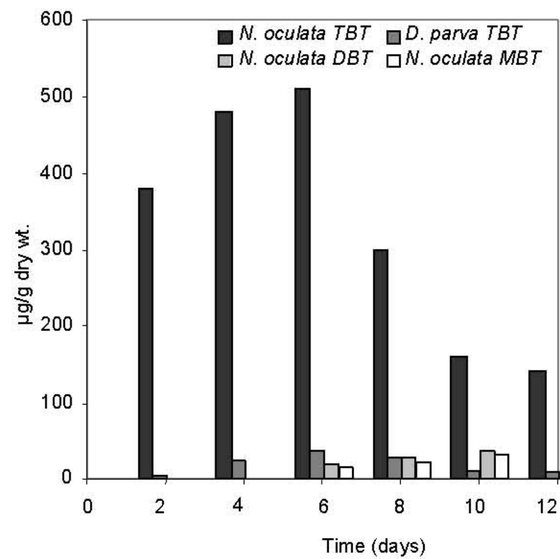

Fig. 3: The content of TBT, DBT and MBT on the wall of Nannochloropsis oculata and *Dunaliella parva*

detected in the culture medium after two days of culturing, while MBT was detected in the culture medium after 4 days of culturing.

Results recorded in Fig. 3 show the various butyltin species associated with the extracellular surface of the two tested algae. The results cleared that the extracellular rigid wall plays an important role for biosorption of TBT. It was noted that, when TBT was added to the culture of *N. oculata* an instant adsorption was noticed reaching maximum at the 6th day of culturing, then abruptly, decreased till the end of the experiment. On the contrary, in case of the wall-less *D. parva* a slight increase in the adsorption of TBT was noticed reaching maximum at the 6th day, then it dropped gradually at the end of the experiment. This means that the phenomenon of wall adsorption could be neglected for this wall-less alga.

The profile of the intracellular TBT species (Fig. 5) in *D. parva* species cleared that TBT was continuously accumulated in the cell interior from the first 2 days to the 6 day of culturing and a maximum of 35 µg TBT/g dry wt. For *N. oculata* (Fig. 4) the cellular content of TBT was first detected at the 2nd day of culturing then increased gradually till the 8th day of culturing reached nearly 90 µg/g dry wt.

The debutylated products of TBT in the cell interior of the two tested algae demonstrated the ability of the wall-less *D. parva* in degrading TBT than the walled *N. oculata*. For *D. parva* DBT and MBT appeared sequentially on days 2 and 4 respectively and a maximum of 28 µg DBT /g dry wt of cells were recorded at day 6 of

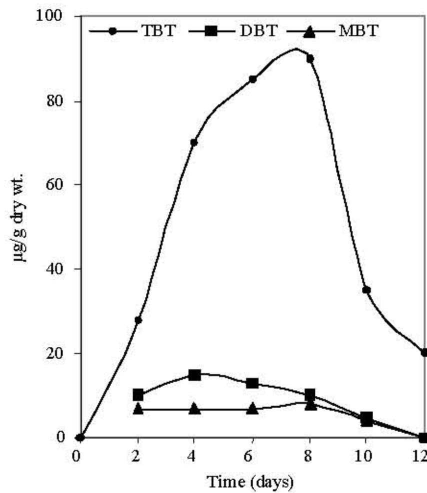

Fig. 4: The intracellular content of TBT, DBT and MBT of the sublethal TBT of *Nannochloropsis oculata*

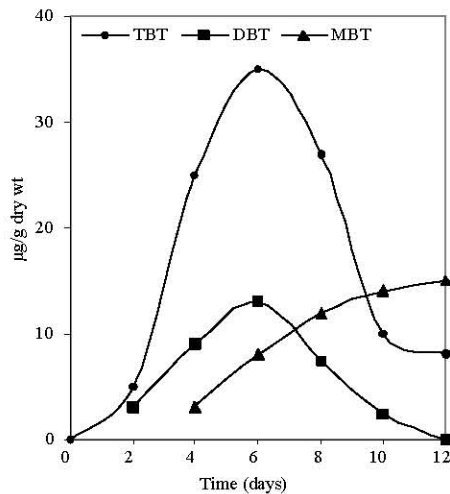

Fig. 5: The intracellular content of TBT, DBT and MBT of the sublethal TBT of *Dunaliella parva*

culturing. The decrease of DBT level from day 6 on wards was concaminant with an increase in the MBT level (Fig. 5), which continued until the end of the experiment (15 µg/g dry wt). However, the walled celled *N. oculata* contained very small amounts of DBT and MBT throughout the study (Fig. 4).

DISCUSSION

The obtained results cleared that both types of the two tested algae have the ability to reduce the concentration of TBT in the medium during the first days of inoculation. The biosorption efficiency of the cell walled algae could be considered as physical in nature [7,21] and could be considered as the major mechanism in localizing TBT in cell wall exterior in the first few days of culturing.

The anionic nature of the algal cell wall provided ligand sites for adsorption of TBT cations. This idea goes parallel with those obtained by Avery *et al.* [13] and Tsang *et al.* [7]. They found that a significant amount of TBT could be adsorbed on cell wall of dead algal cells. However, the main way of TBT absorption was through the active one. This may attributed to the reason of accumulation of TBT inside the cells of the wall-less alga *D. parva* and consequently has the ability of degrading TBT higher than walled celled algae. The transport of TBT into intracellular space may be, therefore, be facilated by some ion channels or carriers in the cell membrane [7, 22]. However, in both cases TBT was accumulated to a peak level and then decreased as a result of its degradation to DBT and MBT. The gradual reduction of TBT and the sequential occurrence of DBT and MBT in medium cultured with algal cells strongly indicated that both tested algae had the ability to degrade TBT. A number of reports indicated that the rate of MBT degradation to inorganic tin was much lower than that of TBT and DBT [10]. The stepwise debutylation of TBT was believed to involve a cascade of enzymatic reaction [7, 23].

The sequential appearance of the intracellular DBT on day 2 and MBT on day 4 in *D. parva* may provide direct evidence on its ability in debutylating TBT to DBT and in turn DBT to MBT through some metabolic pathways. The debutylation of TBT to DBT and MBT was correlated to the presence of special TBT metabolizing enzymes [7]. The drop of intracellular DBT with concomenant increase of MBT from day 6 to the end of the experiment in *D. parva* may be correlated to the fact that, this alga perhaps posses special enzymes for debutylating DBT to MBT. This idea is in harmony with those of Tsang *et al.* [7, 24].

The ability in degrading TBT was considered to be species dependant. Upon exposure to TBT, the first phase would be the rapid biosorption of TBT onto the cell surface. The TBT then accessed to the cell interior through diffusion or via some ion channel as proposed by St-Louis *et al.* [22]. The ability in debutylating TBT to less toxic forms of DBT and MBT correlated well with the tolerance ability of the algal species. However, Gadd *et al.* [25] and Avery *et al.* [13] reported that some microorganism could prevent the entry of TBT into their cells by excreting sorbent to the cellular surface to biosorb TBT. The ability of some microorganism to resist

toxicity of TBT may be due to their ability to transfer TBT into a much less toxic metabolite. Luan *et al.* [26] found that alginate immobilized *Chlorella vulgaris* was able to continuously remove and degrade TBT even at the highest contamination level.

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