



# Removal of tributyltin (TBT) from wastewater by microalgae

N. F. Y. Tam, A. Chong & Y. S. Wong

<sup>1</sup>*Department of Biology and Chemistry,  
City University of Hong Kong, Hong Kong, China.*

## Abstract

The biosorption and biodegradation of tributyltin (TBT) by free cells of a resistant microalgal species, *Chlorella sorokiniana* at two different densities,  $1 \times 10^6$  cells  $\text{ml}^{-1}$  (low) and  $1 \times 10^7$  (high) were investigated. TBT in contaminated water was continuously removed by microalgal cells and reached a 54% and 74% removal at the end of 14 days treatment in low and high density cultures, respectively. The pattern of TBT removal in both densities was similar: with the decreases of TBT in water, TBT uptake by algal cells increased, reached a peak and declined to low levels towards the end of the treatment period. In the high density culture, total amounts of TBT uptake, including the adsorption on cell surface and absorption inside the cells, increased from 109 to 159  $\mu\text{g TBT}^+$  from days 1 to 7, then declined to 95  $\mu\text{g TBT}^+$  at day 14. The proportions of TBT adsorbed on the cell surface decreased while that in the intracellular fraction increased with time, suggesting that the adsorbed TBT moved gradually into the cells for stepwise debutylation. The two degradation products, DBT (dibutyltin) and MBT (monbutyltin), started to appear in the intracellular fraction from day 3 and day 7 onwards in high and low density cultures, respectively, with more MBT than that of DBT. These results indicate that *C. sorokiniana* was not only capable of removing TBT from contaminated water, TBT was absorbed and degraded into DBT which was then rapidly metabolized to MBT by intracellular enzymes.

## 1 Introduction

The widespread use of organotins as biocides has led to serious environmental contamination because of their detrimental effects on aquatic organisms. The

## 262 Water Pollution VII: Modelling, Measuring and Prediction

most toxic organotin is tributyltin (TBT) and their toxicity reduces when degraded into di- and mono-substituted organotin compounds, and the least toxic one is the inorganic tin. Therefore, one possible way to remove TBT contamination is to adsorb, absorb and biodegrade them into less toxic metabolites by a cascade of enzymatic reactions. Microorganisms such as microalgae, fungi and bacteria are expected to be able to biosorb and degrade organotins [1, 2]. Harino *et al.* [3] reported that bacterial species in the aquatic environment could debutylate TBT. The efficiency and ability of microalgae to remove organotin is uncertain as some microalgae were found to have the ability to biosorb and biodegrade organotins [4, 5] but some species failed to do so [6]. Moreover, the treatment efficiency often depends on the amounts of biomass employed. The present study therefore aims to compare the low and high cell densities of *Chlorella sorokiniana*, a TBT resistant isolate from East Lake, Wuhan, China on biosorption, bioaccumulation and biodegradation of TBT. The distribution of TBT and its degradation products in the medium, on the cell surface and inside the cell during 14 days treatment was also investigated.

## 2 Materials and methods

### 2.1 Mass algal culture

Stock culture of *Chlorella sorokiniana* was mass cultured in 10 l plastic vessels containing Bristol medium with pH maintained at 6.8-7.2. One liter of the medium consists 10 ml of each of the following chemicals: 25g/l NaNO<sub>3</sub>, 7.5g/l K<sub>2</sub>HPO<sub>4</sub>, 17.5g/l KH<sub>2</sub>PO<sub>4</sub>, 11.8g/l MgSO<sub>4</sub>·7H<sub>2</sub>O, 2.5g/l NaCl, 2.5g/l CaCl<sub>2</sub> and trace element (a mixture of microelements). The cultures were aerated with 0.2mm filtered air, incubated in an environmental chamber illuminated with cool white fluorescence lights at a light intensity of 175µmol/s/m<sup>2</sup> with a light/dark cycle of 16/8 h, and temperature was maintained at 25±1°C. After one week of culture, algal cells at their log phase were harvested by centrifugation at 5,000 rpm for 10 min. The algal cell residues were washed, resuspended in deionized water and the cell number in the concentrated culture was counted.

### 2.2 Biosorption and Biodegradation of TBT

A total of 60 sterilized 2 l conical flasks, each containing 1.5 l of the culture medium, were prepared. Fifty-four flasks were spiked with an appropriate amount of stock TBTCl<sub>2</sub> solution (100mg/l) aseptically to give an initial TBT<sup>+</sup> concentration of 100µg/l that was the sub-lethal concentration of this species [7]. These 54 flasks were divided into three groups, 18 were inoculated with the concentrated algal culture at a low initial cell density of 1×10<sup>6</sup> cells/ml, and the other 18 were inoculated with algae at a high density of 1×10<sup>7</sup> cells/ml, and the remaining 18 flasks were used as the blank, without any algal inoculation for monitoring any abiotic TBT loss. The remaining 6 flasks without any TBT

addition were used as control algal flasks, three with low cell density inoculation and three with high cell density. The flasks were shaken on a rotary shaker at 100 rpm in an environmental chamber described above. The growth in each algal group was monitored by counting the number of viable cells using an improved Neubauer haemocytometer after staining the cells with neutral red. Duplicate counts were made for each sample and triplicate samples were collected for each algal group. Triplicate flasks from each TBT treated group were retrieved at 5 min, 1, 3, 7, 10 and 14 days of treatment to determine the concentrations of residual butyltins in the medium and the distribution in algal cells following the method described by Tam *et al.* [5].

### 2.3 Determination of butyltins

The algal suspension collected was centrifuged at 5,000 rpm for 10 min at 4°C. The supernatant was used for determination of the residual butyltins in the medium. The algal pellets were shaken with 10 ml 1M HCl at 100 rpm for 1 h, centrifuged at 4,000 rpm for 10 min, and the supernatant was collected. This step was repeated, and the combined supernatant represented the cell surface fraction and accounted for the butyltins adsorbed on algal cell walls (i.e. extracellular uptake). The residual butyltin remained in the medium and that adsorbed on cell walls were determined according to Tam *et al.* [5]. The samples were acidified for 1 h. A mixture of 10mg/l triphenyltin chloride (used as an internal standard), 0.5 ml tropolone (1% in methanol) and 100 mg ascorbic acid were added, and extracted through a tropolone-Sep-PAK C18 cartridge. Diethyl ether (20 ml) was then added, and the extract was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, and derivatized by Grignard reagent. The reaction mixture was acidified, dried and purified with Ultra-clean Florisil (Supelco, USA) and diluted to 1 ml with 10% diethyl ether in hexane. The quantities and speciation of butyltins were measured using a capillary gas chromatography equipped with a flame photometric detector (GC-FID, Hewlett Packard 5890 Series II) as described by Tam *et al.* [5].

To obtain the intracellular fraction, the remaining cell pellet was suspended in 0.5 ml 0.1M HCl and beaten three times by a mini-bead beater (Biospec products) at 42 rpm for 1 min. Triphenyltin chloride (10mg/l) was added as an internal standard, and the mixture was extracted three times with 10 ml 0.25% tropolone in diethyl ether. The organic phases were combined and dried, then undergone derivation and GC determination as mentioned above.

## 3 Results and discussion

### 3.1 Removal of TBT by low and high densities of *C. sorokiniana*

The growth of *C. sorokiniana* at both low and high cell densities was not affected by the presence of TBT in the medium and no significant differences in cell number were found between the TBT treated and control cultures (Figure 1).

264 *Water Pollution VII: Modelling, Measuring and Prediction*

The  $\text{TBT}^+$  concentrations in the blank flasks (with TBT added but without algal inoculation) remained relatively constant, fluctuated between 87 to 105  $\mu\text{g}/\text{l}$  throughout the 14 days treatment (Figure 2) and only trace amounts of DBT and MBT were detected, indicating that TBT was stable against photochemical and other abiotic degradation within the experimental period. Photolysis has been shown to be slow with a half-life longer than 89 days in the environment and is not a significant process to remove TBT [8]. The biological degradation of TBT is much faster and more applicable for TBT removal than photolytic cleavage.

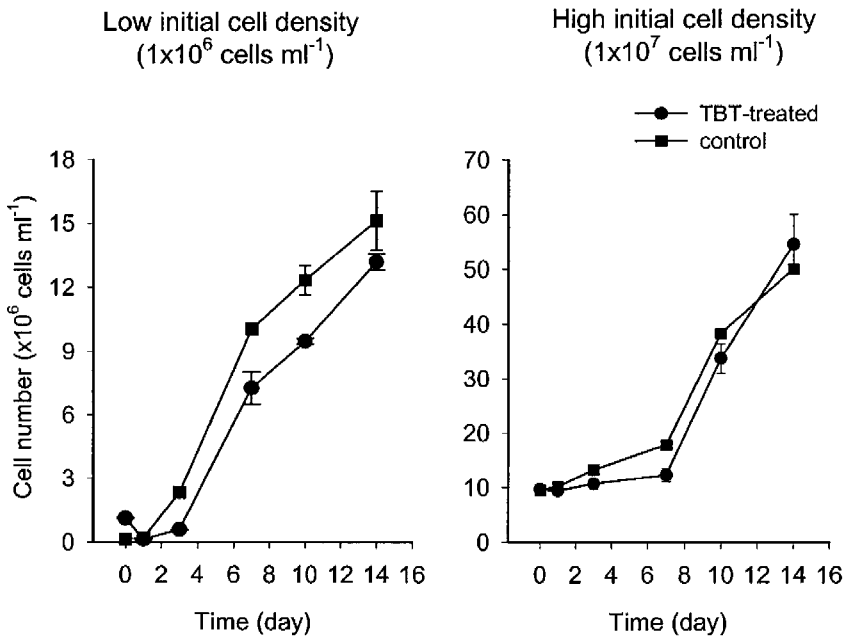


Figure 1: Growth of control and TBT treated *S. sorokiniana* under high and low cell densities (mean and standard deviation of triplicates are shown)

The  $\text{TBT}^+$  concentrations in the medium treated by *C. sorokiniana* showed a rapid decline in the first 5 minutes of treatment especially at high initial cell density. The removal percentages within 5 minutes were 14 and 36% in low and high cell density cultures, respectively. These results indicate that the initial TBT removal was mainly due to physico-chemical adsorption on the cell wall, and the more the cell wall binding sites due to larger cell numbers, the more the adsorption. Avery *et al.* [1] stated that the first uptake of organotin in microorganisms occurs rapidly, primarily through adsorption on the cell surface, with little or no intracellular accumulation. The adsorption process is generally passive or non-metabolic, including ion exchange, adsorption, complexation, precipitation and crystallization with carboxyl, phosphate or other function groups on the cell surface. The anionic nature and organic molecules of the algal

cell wall provide ligand sites for the adsorption of TBT cations. Saint-Louis *et al.* [9] reported that the adsorption of TBT by exudates coating on the cell wall of a microflagellate, *Pavlova lutheri* was 10-20%. The high cellulose and lipid content in microalgal cell walls could have strong binding affinity to hydrophobic chemicals [10]. Sijm *et al.* [11] suggested that high algal densities might have a larger lipid pool and have more exudates excreted for binding organic contaminants.

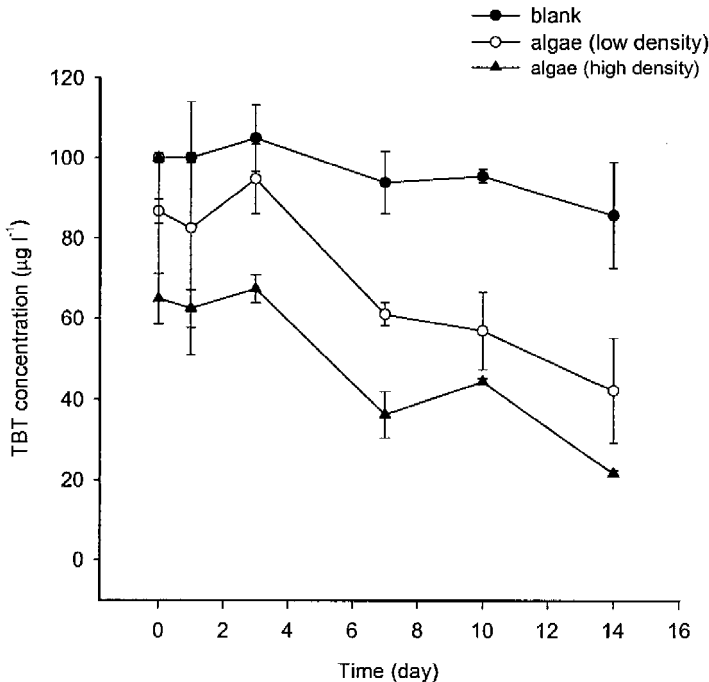


Figure 2: Concentrations of TBT remained in liquid media.

After an initial drop, the TBT concentrations in the medium remained relatively stable for three days, and a gradual drop occurred thereafter. At the end of the experiment, the TBT<sup>+</sup> concentrations were around 40 and 20µg/l in low and high cell density cultures, respectively (Figure 2). It is obvious that the efficiency of TBT removal by low cell density was 20% less than that by high cell density at the same treatment time. These suggest that not only the initial adsorption, more cells would also enhance the degree and rate of TBT accumulation and degradation by *C. sorokiniana*.

### 3.2 Distribution and biodegradation of TBT

As the concentrations of TBT in the medium decreased, the amounts of TBT adsorbed on the algal surfaces increased and such increase was more rapid in high than that in low cell density cultures (Figure 3). The peak adsorption was observed in day 7 with a value of  $477\mu\text{g TBT}^+/\text{g}$  of cell surface materials in low density culture while the peak in high density one was recorded in day 1 with more than  $521\mu\text{g TBT}^+/\text{g}$  adsorbed on the cell surface. After the peak, the concentrations of TBT adsorbed decreased and reached very low levels (around  $70\mu\text{g/g}$ ) from days 10 and 7 onwards in low and high density cultures, respectively.

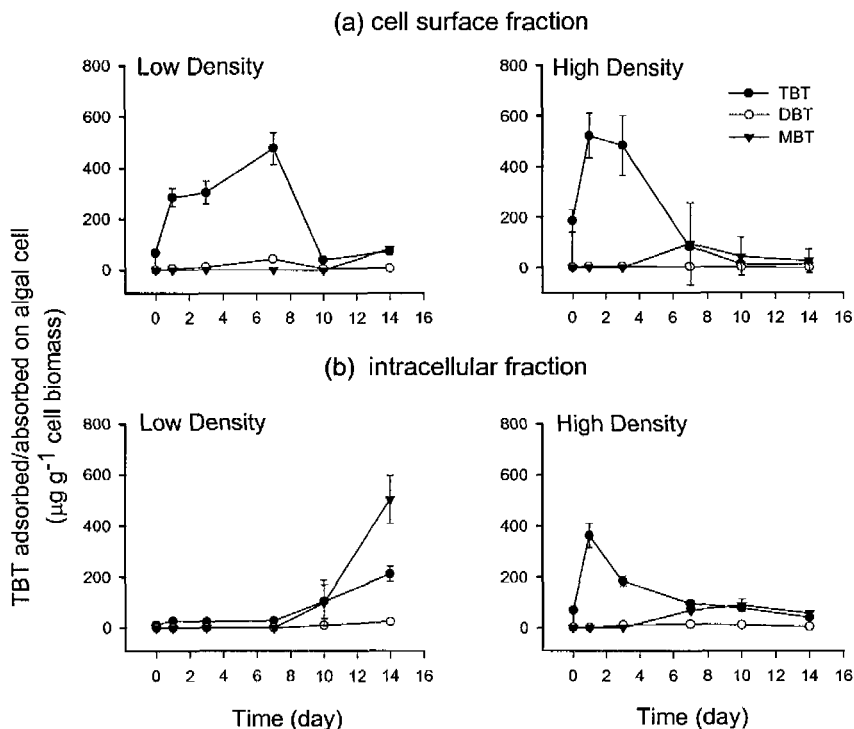


Figure 3: Concentrations of TBT, DBT and MBT distribution in *C. sorokiniana*.

Not only adsorbed on the cell surface, TBT was also absorbed and accumulated inside the cells. The sharp increase in intracellular TBT concentration was observed between days 10 to 14 in low cell density culture while the accumulation was more obvious in the first 3 days in high density treatment. Figure 4 reveals that over 95% of the TBT uptake by algae was adsorbed on the cell surface in the first 5 minutes to 7 days and the percentages

dropped to 22% in day 14 in low density culture. On the contrary, the percentages accumulated in the intracellular fraction increased significantly from day 10 onwards. The high density treatment followed a similar but less obvious distribution pattern as TBT started to accumulate inside the cells earlier and a larger percentage of TBT was accumulated intracellularly (more than 50% of total TBT uptake by algae) than that in low cell density culture at least in the first week of treatment. At the end of the experiment, the amount of TBT<sup>+</sup> uptake by low density cells was 29.9 $\mu$ g (equivalent to 12.4 $\mu$ g Sn), less than half of that found in high density culture (Table 1). The transport of TBT into intracellular space *via* ion channels or carriers in the cell membrane is a second phase of uptake and occurs slowly. Previous studies show that TBT in microbial cells was accumulated to a peak as time proceeded but then decreased as a result of its degradation to DBT and MBT [2, 4].

Table 1. Mass balance of Sn atom showing the degree of TBT removal and degradation by *C. sorokiniana* at two cell densities ( $\mu$ g Sn biodegraded =  $\mu$ g Sn added to medium -  $\mu$ g Sn remained in medium -  $\mu$ g Sn uptake in cells as TBT; the initial Sn added was 106.39 $\mu$ g Sn; data in brackets show the percentage of the added TBT; mean value of triplicates was used for calculation).

Time and Density	$\mu$ g Sn remained in medium	$\mu$ g Sn uptake in cells as TBT	$\mu$ g Sn degraded by cells	$\mu$ g Sn adsorbed and accumulated in cells	
				As DBT	As MBT
Day 1					
Low	98.34 (92.4%)	5.64 (5.3%)	2.41 (2.3%)	0.14	ND
High	50.85 (47.8%)	46.39 (43.6%)	9.15 (8.7%)	0.32	ND
Day 7					
Low	78.88 (74.1%)	12.70 (11.9%)	14.81 (13.9%)	1.36	14.10
High	30.62 (28.8%)	37.68 (47.3%)	38.09 (35.8%)	4.03	36.99
Day 14					
Low	45.44 (42.7%)	12.40 (11.7%)	48.55 (45.6%)	1.71	41.95
High	28.51 (26.8%)	25.24 (23.7%)	52.72 (49.6%)	7.81	46.63

The present study shows that in high cell density culture, the amount of TBT uptake by algal cells decreased with the treatment time, from 114 $\mu\text{g}$  TBT<sup>+</sup> (equivalent to 46 $\mu\text{g}$  Sn) in day 1 to 62 $\mu\text{g}$  TBT<sup>+</sup> (equivalent to 25 $\mu\text{g}$  Sn) in day 14 (Table 1). This suggests that some TBT absorbed might have been degraded to DBT and MBT by stepwise debutylation. The intracellular increases of the degradation products, in particular MBT were obvious from day 7 onwards. In low cell density treatment, little TBT accumulation was found between days 7 and 14, intracellular accumulation of DBT and MBT was not found until day 10 but their concentrations increased rapidly towards the end of the experiment and were higher than that on the cell surface (Figure 3). These results indicate that TBT absorbed were biodegraded and intracellular degradation was far more significant than the extracellular process. Similar findings were reported by Seligman *et al.* [12] that an algal strain, *Ankistrodesmis falactus* was capable of adsorbing, accumulating TBT oxide and degrading more than 50% TBT oxide to dibutyl derivatives or monobutyl derivative or even to inorganic tin under axenic culture condition. Tsang *et al.* [4] showed that 27 and 41% of the added TBT were recovered as DBT and MBT, respectively in *C. vulgaris* cultures.

In both surface and intracellular fractions, MBT concentrations were higher than that of TBT and DBT at the end of the experimental period (Figure 3). TBT in microorganisms is degraded into less toxic metabolites by two phases. The first phase reactions involve the cytochrome P-450 dependent monooxygenase system which hydroxylates TBT to alpha-, beta, gamma-, and delta-hydroxyldibutyltin derivatives, and the phase two reactions conjugate sugars or sulfate to hydroxyldibutyltin, and these polar conjugates are rapidly eliminated from the organisms [4]. Tsang *et al.* [4] further proposed that the activity of E2 enzyme catalyzing the debutylation reaction from DBT to MBT was faster than the conversion of TBT to DBT by the E1 enzyme, therefore DBT formed would be rapidly metabolized to MBT with little accumulation in algal cells.

The mass balance of Sn atoms shows that in the first week, more TBT degradation and MBT accumulation were found in high than low density cultures, however, no significant differences were observed at the end of the 14 days treatment (Table 1). At the end of the 14 days treatment by high density cultures, around 24% of the added TBT was accumulated, 50% was degraded into MBT with minor accumulation of DBT, and intact TBT remaining in the medium was 27%. More TBT was remained intact in the medium with less TBT accumulated in low density treatment although the degradation percentage was comparable between high and low density cultures. These results suggest that the efficiency of *Chlorella sorokiniana* to uptake and degrade TBT depended on the cell density, more cells led to quicker degradation. The percentage of the added TBT (100 $\mu\text{g}/\text{l}$ ) remained intact in the medium was smaller and the degradation products (DBT and MBT) accumulated in *C. sorokiniana* cells were larger than



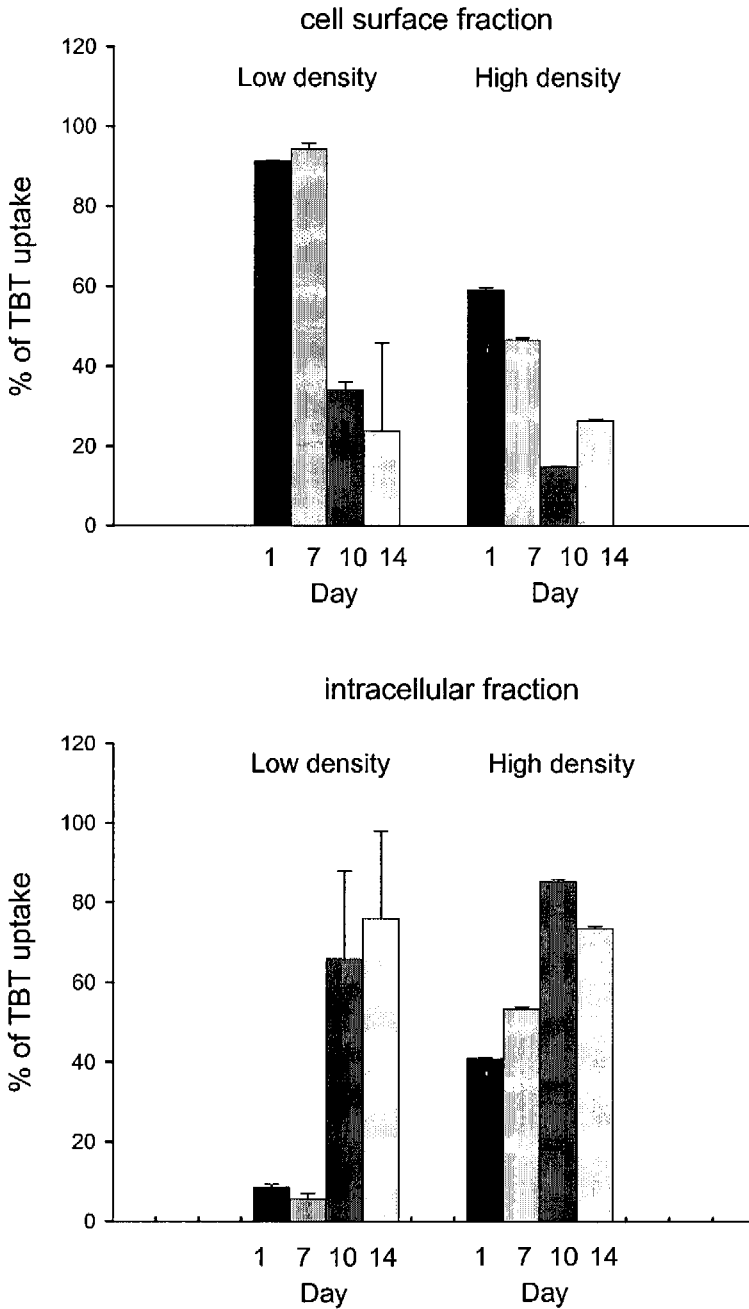


Figure 4: Relative proportion of TBT adsorbed and absorbed by *C. sorokiniana* at low and high densities.



those reported by previous researchers. For instance, Reader and Pelletier [13] reported that 30% of the initial TBT (1 $\mu$ g/l) was left intact in the medium, 50% recovered in the culture as dibutyltin, and 15% incorporated in *Skeletonema costatum* cells. Similarly, the initial TBT (0.4 $\mu$ g/l) left in the medium with the same algal species was 25%, the recovery of the initial TBT as DBT was 50%, and 25% metabolized into hydroxylated by-products by *S. costatum*, while only 12% DBT was found in *Dunaliella* cultures [6]. These suggest that *C. sorokiniana* examined in the present study was more effective in removing and degrading TBT from the medium than other microalgal species.

## References

- [1] Avery, S.V., Codd, G.A. & Gadd, G.M., Biosorption of tributyltin and other organotin compounds by cyanobacteria and microalgae. *Applied Microbiology and Biotechnology*, **39**, pp. 812-817, 1993.
- [2] Kawai, S., Kurokawa, Y., Harino, H. & Fukushima, M., Degradation of tributyltin by a bacterial strain isolated from polluted river water. *Environmental Pollution*, **102**, pp. 259-263, 1998.
- [3] Harino, H., Fukushima, M., Kurokawa, Y. & Kawai, S., Susceptibility of bacterial populations to organotin compounds and microbial degradation of organotin compounds in environmental water. *Environmental Pollution*, **98**, 157-162, 1997.
- [4] Tsang, C.K., Lau, P.S., Tam, N.F.Y. & Wong, Y.S., Biodegradation capacity of tributyltin by two *Chlorella* species. *Environmental Pollution*, **105**, 289-297, 1999.
- [5] Tam, N.F.Y., Chong, A.M.Y. & Wong, Y.S., Removal of tributyltin (TBT) by live and dead microalgal cells. *Marine Pollution Bulletin*, **45**, 362-371, 2002.
- [6] Lee, R.F., Valkirs, A.O. & Seligman, P.F., Importance of microalgae in the biodegradation of tributyltin in estuarine waters. *Environmental Science and Technology*, **23**, 1515-1518, 1989.
- [7] Tam, N.F.Y., Wong, Y.S. & Chong, A.M.Y., Toxic effect of tributyltin (TBT) on different microalgal species. *Algae and their Biotechnological Potential*, eds. F. Chen & Y. Jiang, Kluwer Academic Publishers: Netherlands, pp. 181-194, 2001.
- [8] Stewart, C. & deMora, S.J., A review of the degradation of tri(n-butyl)tin in the marine environment. *Environmental Technology*, **11**, 565-570, 1990.
- [9] Saint-Louis, R., Pelletier, E., Marsot, P. & Fournier, R., Distribution and effects of tributyltin chloride and its degradation products on the growth of the marine alga *Pavlova lutheri* in continuous-culture. *Water Research*, **28**, 2533-2544, 1994.
- [10] Swackhamer, D.L. & Skoglund, R.S., Bioaccumulation of PCBs by algae: kinetics versus equilibrium. *Environmental Toxicology and Chemistry*, **12**, 831-838, 1993.



- [11] Sijm, D.T.H.M., Broersen, K.W., deRoode, D.F. & Mayer, P., Bioconcentration kinetics of hydrophobic chemicals in different densities of *Chlorella pyrenoidosa*. *Environmental Toxicology and Chemistry*, **17**, 1695-1704, 1998.
- [12] Seligman, P.F., Valkirs, A.O., Stang, P.M. & Lee, R.F., Evidence for rapid degradation of tributyltin in a marina. *Marine Pollution Bulletin*, **19**, 531-534, 1988.
- [13] Reader, S. & Pelletier, E., Biosorption and biodegradation of butyltin compounds by the marine diatom *Skeletonema costatum* and the associated bacterial community at low temperature. *Bulletin of Environmental Contamination and Toxicology*, **48**, 599-607, 1992.

