APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2003; **17**: 3–8
Environment, Biology and Toxicology AOC
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.394

Review

Biodegradation of tributyltins (organotins) by marine bacteria

S. K. Dubey* and U. Roy

Laboratory of Bacterial Genetics and Environmental Biotechnology, Department of Microbiology, Goa University, Taleigao Plateau, Goa 403206, India

Received 18 January 2002; Accepted 12 September 2002

Many marine bacterial strains have an inherent capability to degrade toxic organotin compounds, especially tributyltins (TBTs), that enter into the environment in the form of insecticides, fungicides and antifouling paints as a result of anthropogenic and industrial activities. Significant degradation of these compounds in the ambient environment may take several years, and it is necessary to consider methods or strategies that can accelerate the degradation process. There have been few demonstrations of biological degradation of these organotin biocides exclusively in laboratory-scale experiments. Compared with the few bench-scale degradation processes, there are no reports of field-scale processes for TBT bioremediation, in spite of its serious environmental threat to non-target organisms in the aquatic environment. Implementation of field-scale biodegradation of TBT requires inputs from biology, hydrology, geology, chemistry and civil engineering. A framework is emerging that can be adapted to develop new processes for bioremediation of toxic environmental wastes. In the case of TBT bioremediation, this framework incorporates screening and identification of natural bacterial strains, determination of optimal conditions for growth of isolates and TBT degradation, establishment of new metabolic pathways involved in TBT degradation, identification, localization and cloning of genes involved in degradation and in TBT resistance, development of suitable microbial strains using genetic manipulation techniques for practical applications and optimization of practical engineering processes for bioremediation of organotin-contaminated sites. The present review mainly addresses the aspect of TBT biodegradation with special reference to environmental sources of TBT, chemical structure and biological activity, resistant and degrading bacterial strains, possible mechanisms of resistance and degradation and the genetic and biochemical basis of TBT degradation and resistance. It also evaluates the feasibility and potential of natural and genetically modified TBT-degrading bacterial strains in field-scale experiments to bioremediate TBT-contaminated marine sites, and makes recommendations for more intensive and focused research in the area of TBT bioremediation mediated by marine bacterial strains. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: biodegradation; tributyltin (TBT); microorganism; bioremediation; resistance mechanisms; toxicity.

ORGANOTINS IN THE ENVIRONMENT

Organotin compounds remained of purely scientific interest for a long time, since their discovery around 1850. Though the first mention of a practical application of organotin compounds was made in a patent taken out in 1943, which indicated their potential in antifouling systems,[1] commercial production only started in 1960s. All organotin compounds are toxic, but the effect varies according to the number and the type of organic moiety present, as propyl- and butyl-group-bearing organotins are more toxic to fungi and bacteria.[2] Extensive use of organotins worldwide has

*Correspondence to: S. K. Dubey, Laboratory of Bacterial Genetics and Environmental Biotechnology, Department of Microbiology, Goa University, Taleigao Plateau, Goa 403206, India.
E-mail: dubey_santosh1@rediffmail.com
Contract/grant sponsor: Department of Ocean Development, India.

provoked scientific interest on the toxic effect of organotin compounds on aquatic and terrestial biota.[3–5] Tributyltin (TBT) has been in use as a paint additive since the 1970s to prevent bio-fouling on ship hulls, marine platforms and fishing nets. By the mid 1980s, researchers in France and the UK had confirmed that TBT present in antifouling paints adversely affects non-target organisms. In 1982, France was the first country to ban the use of organotin-based antifouling paints on boats less than 25 m long.[6,7] Similar regulations have also been imposed in North America, Australia, New Zealand, South Africa, Hong Kong and most European countries since the late 1980s.[8–14] Subsequently, worldwide monitoring programmes have shown reduced concentrations of TBT in the water column, sediments and tissues of marine animals. The International Maritime Organization (IMO) has repeatedly expressed concern about the harmful effects of TBT-based paints.[12] It has also been shown that TBT may be responsible for the weakening of oyster and mussel shells, as well as of retardation of growth of various species of aquatic snails.[15,16] Two widely published events in the 1980s, viz the near-collapse of oyster farming in Arcachon bay, Western France, and the demise of populations of dogwhelk, *Nucella lapillus*, close to boating activity in southwest England, have been attributed to severe TBT contamination. These studies resulted in a number of surveys of TBT pollution worldwide and also indicated that the problem was global.[12,17–20] TBT concentrations in the aquatic environment have been monitored for many years at many locations throughout the world, including the North Sea, Black Sea, Atlantic Ocean, Pacific Ocean and Japanese waters.[7,12,21–23] Noticeable concentrations of organotins reported so far include: 38 μg g^{-1} TBT in Suva Harbour, Fiji; 10.780 ng g^{-1} Hexyltin in Vancouver, Canada; 518 ng g^{-1} TBT in Boston Harbour, USA; 400 ng g^{-1} TBT in Lake Lucerne, Switzerland; and up to 380 ng g^{-1} TBT in Puget Sound, USA.[9,10,18] The IMO has passed a resolution to ban the application of TBT-based antifouling paints on ships and boats and has also proposed to establish a mechanism to prevent the potential future use of other harmful substances in antifouling systems.[13] Triorganotins, such as tributyltin oxide (TBTO), tributyltin chloride (TBTCl), triphenyltin chloride (TPTCl), tributyltin fluoride (TBTF), tributyltin hydroxide (TBTH), tributyltin naphthenate (TBTN) and tris(tributylstannyl) phosphate (TBTP), are used extensively as biocides in antifouling paints on ship hulls, boats and docks, as slimicides in cooling towers, as fungicides, bactericides and insecticides, as preservatives for wood, textiles, papers and leather, as stabilizing material in PVC pipes, electrical equipment and as catalysts for synthesis of polyurethane foam and silicone rubber.[24–26] Trisubstituted organotins have wide-ranging toxicological properties, and their biocidal uses have been reported to have detrimental environmental impacts.[27] In the UK, under the Control of Pollution Act, 1974, the retail sale of organotin paints was restricted to co-polymer paints containing <7.5% tin and free association paints containing <2.5% tin in the dry film.[8] During the 1990s, the USA alone produced 10000 t of organotin compounds each year.[28] Recent estimates show that the annual world production of organotins may be close to 50000 t per year.[27] Commercial ships, in particular, consume about 75% of total TBT used as antifouling paints.[29] In Suva Harbour, Fiji, water blasting of relatively large vessels has caused severe contamination of near-shore sediments and shellfish. A British survey revealed that unregulated dry dock practices clearly result in the release of large quantities of TBT into marine environment.[9] Non-point sources of environmental exposure include the discarding and sanitary landfill disposal of plastic and the direct release of biocides to the aquatic and marine environments. Other dissipative uses of organotins that pose potential risk to humans include PVC food wrappings, bottles and rigid potable water pipes, although long-term human health hazards due to low-level exposure to organotins are not known. Toxic metal cycling in the environment, including biomethylation of inorganic tin by naturally occurring bacteria, is also of concern.[30,31] *In situ* measurement of TBT-based antifouling paint leachates have shown that TBT is the principal compound released in water. It has been shown that different forms of TBTs, such as the hydroxide, chloride, and various carboxylate forms are released into the aqueous environment from different types of paint as a result of leaching.[24]

CHEMISTRY OF TBT (ORGANOTIN) COMPOUNDS

In view of the diversity of organotins used industrially, knowledge of their environmental chemistry is of fundamental importance, and some aspects have already been reviewed.[30] The TBT compounds are a subgroup of the trialkyl organotin family. It is interesting to note that the alkyls tend to be more toxic than the aryls, and that triorganotins are more toxic than di-, mono- or tetra-organotins. Generally, the toxicity of the organotin is influenced more by the alkyl substituents than the anionic substituents. Progressive introduction of organic groups to the tin atom in any member of the R_3SnX_{4-n} series produces maximal biological activity against all species, when $n = 3$, for R_3SnX.[3,4,32,33] Generally, trisubstituted (R_3SnX) organotins, where R = butyl or phenyl, are more highly toxic than di- and mono-substituted organotin compounds, and the anion (X) has little influence on the toxicity.[34,35] It is interesting to note that they can provide antifouling cover for 5 years or more and have been acclaimed widely as the most effective antifoulants ever devised. TBT in such paints is chemically bonded in a copolymer resin system via an organotin–ester linkage, but there is a slow and controlled release of the biocide, as the link becomes hydrolysed when sea water comes into contact with the paint surface.[12]

BIOLOGICAL ACTIVITY OF ORGANOTINS

Though tin in its inorganic form is considered to be non-toxic, the toxicological pattern of the organotin compounds is complex.[20] TBT, tripropyltin and triphenyltin are highly effective biocides against several marine fouling organisms, including bacteria and fungi, whereas tricyclohexyltin compounds exhibit miticidal properties. In general, organotin toxicity to microbes decreases in the following order: $R_3SnX > R_2SnX_2 > RSnX_3 > R_4Sn$. Since, microorganisms accumulate organotins in the cell wall envelope by a non-energy requiring process, organotins such as tripropyl-, tributyl- and triphenyl-tin seem to be highly toxic to bacteria and fungi.[36–38] It is interesting to note that increased total surface area and lipid solubility of the trisubstituted tin correlates well with the toxic effects observed and confirms that triorganotins exert toxicity through their interaction with membrane lipids. It has been reported that organotin compounds are toxic to both Gram negative and Gram positive bacteria but triorganotins are more active towards the Gram positive bacteria than towards Gram negative bacteria. Among the trialkyltin series, the most active compounds inhibiting growth of the Gram positive species at 0.1 mg l^{-1} belong to the type R_3SnX. Gram positive bacteria are less sensitive to triethyl and tripropyltin acetate or chloride than Gram negative bacteria, whose growth is inhibited at concentrations of 20–50 μg ml^{-1}. TBTCl and TBT acetate have stronger growth inhibitory effects on Gram positive bacteria than on Gram negative bacteria.[39,40]

TBT is a membrane-active lipophilic compound known to exhibit the same inhibitory mechanisms in bacteria as seen in mitochondria and chloroplasts by acting as an ionophore-facilitating halide–hydroxyl ion exchange by interfering with the energy transduction apparatus. In addition, TBT can inhibit a variety of energy-linked reactions in *Escherichia coli*, including growth, solute transport, biosynthesis of macromolecules and activity of transhydrogenase.[33] Boopathy and Daniels[28] have also tested the toxic effects of several organotins and tin chloride on the methanogenic bacteria *Methanococcus thermolithotrophus*, *Methanococcus deltae* and *Methanosarcina barkeri* 227. These methanogens were strongly inhibited by triethyltin, tripropyltin and monophenyltins generally below the 0.05 mM level. Less inhibition was observed for TBT at 0.1 mM, but there was complete inhibition of growth at 1 mM concentration. Virtually all organotin toxicological studies have been conducted using aerobic microorganisms, viz. bacteria and yeast.[36,37,41–45] In addition, biocidal effects of organotins against other marine fouling organisms viz. algae (*Enteromorpha*, *Ectocarpus* and *Ulothrix*), barnacles, tubeworms and shrimps have also been studied.[46–49]

TBT-RESISTANT BACTERIA

Several reports have been documented on the isolation and characterization of TBT-resistant bacteria from soil, marine and estuarine environments.[25,34,50–55] The isolation and characterization of the TBT-tolerant (-resistant) marine bacterium *Alteromonas* sp. M-1 is the first of its kind. It is interesting to note that addition of TBT to natural sea water specifically enriched TBT-tolerant bacteria.[55,56] These resistant bacteria can tolerate high levels of TBT biocide due to their inherent capability: (i) to transform it into less toxic compounds, viz. dibutyltin (DBT) and monobutyltin (MBT) by a dealkylation mechanism; or (ii) to exclude/effuse these toxicants outside the cell, mediated by membrane proteins; or (iii) to degrade/metabolically utilize it as a carbon source mediated by enzymes; or (iv) to bioaccumulate the biocide without breakdown using metallothionein-like proteins.[56,57]

Little is known about the incidence of organotin resistance in natural microbial populations, or the resistance mechanism by which microorganisms tolerate high levels of organotins.[54] Therefore, the list of potential organotin-resistant bacteria includes *E. coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Serratia marcescens* and *Alcaligenes faecalis*, which are Gram negative, and *Staphylococcus aureus*, *Staphylococcus epidemidis*, *Bacillus subtilis*, *Mycobacterium phlei* and *Vibrio sp.*, which are Gram positive.[26,35,54,56]

DEGRADATION OF TBT BY ABIOTIC AND BIOTIC FACTORS

Organotin degradation involves sequential removal (dealkylation) of alkyl groups from the tin atom, which generally results in a toxicity reduction.[38,57,58] This can be achieved by biotic and abiotic factors, with UV and chemical cleavage being the most important abiotic factors in aquatic and terrestrial ecosystems.[51,58] Although the degradation of organotins has been shown to be mediated by microorganisms, information is still severely limited in relation to the mechanism of degradation the tolerance mechanisms of microbes and their relative significance and also the role of anionic radicals in the degradation process in natural habitats.[35,58,60] Biotic processes have been demonstrated to be the most significant mechanisms for TBT degradation both in soil and in fresh water, marine and estuarine environments.[51,61]

The lack of knowledge on the environmental fate of TBT in coastal waters stimulated research interest on the biodegradation and bioaccumulation of TBT in water columns, sediments and also by higher marine organisms. Environmental surveys from different locations throughout the world have shown that TBT is present in three main compartments of the aquatic ecosystem: the surface microlayer, the water column and the surface layer of the bottom sediments.[24] The result of these studies indicates that TBT can be degraded rapidly in the marine water column to DBT and MBT with a half-life of several days. TBT degradation by photolysis alone proceeds slowly, with a half-life of >89

days.[54] Half-lives from a clean water site (0.03 μg l^{-1} of TBT) were 9 days and 19 days for light and dark treatments respectively,[62] but photolysis probably is not a significant breakdown process for TBT.[24] In the case of TBT present in sediments, a first-order multi-step kinetic model for the sequential degradation of TBT to form DBT, MBT and tin(IV) has been proposed which indicated that the half-life of TBT, DBT and MBT was 2.1 years, 1.9 years and 1.1 years respectively.[63] The principal degradation product in all experiments was DBT with lesser amounts of MBT. Complete mineralization of TBT, measured by the formation of $^{14}CO_2$, proceeded slowly, with a half-life of 50–75 days. Rates of TBT degradation may be influenced by several biotic and abiotic factors, such as the nature and density of microbial populations, TBT solubility, dissolved/suspended organic matter, pH, salinity, temperature and light. Sheldon[64] has reported that ^{14}C-labelled TBTO, TBTF and triphenyltin fluoride in soil was degraded faster in aerobic conditions than in anaerobic conditions. However, persistence does not necessarily equate to a compound being toxic, because it may not be bioavailable.[12] Interaction of microorganisms with organotins is significantly influenced by environmental conditions. In aquatic ecosystems, both pH and salinity can determine organotins speciation/bioavailability and, therefore, biological activity. In one study Pottassium (K^+) release was used as an index of toxicity, as both the rate and the extent of K^+ release was affected by salinity. Increased NaCl concentration reduced the toxic effect of TBT, with the possible effects being due to Na^+ and Cl^- moieties, as well as by possible osmotic responses of the organisms, which included changes in intracellular compatible solute and membrane composition.[44] These environmental factors may also alter selectively the resistance of microorganisms in polluted aquatic systems.[45] Biological and chemical degradation of TBT in marine and freshwater sediments has been reported to be slow,[54] as the half-life of TBT in marine water has been found to be about a week, whereas in sediments it was about 2.5 years.[29] This clearly indicates that sorption of TBT in the silty sediments strongly reduced the bioavailability of the biocide to microorganisms.[65] Because of the low water solubility, TBT preferably binds to suspended organic matter released from marine sediments. The extent of binding to bottom sediments varies with location, organic matter content and particle size.[16]

Abiotic degradation processes have also been put forward as possible pathways for the removal of TBT from soil sediments and water columns, as the Sn—C bond could be broken by four different abiotic processes, viz. UV irradiation, chemical cleavage, gamma irradiation and thermal cleavage.[66] Because gamma irradiation rarely occurs and the Sn—C bond is stable up to 200 °C, gamma irradiation and thermal cleavage have a negligible effect on the environmental breakdown of TBT. Only the near-UV spectrum (300–350 nm) is likely to cause direct photolysis of TBT, and, owing to the low transmittance of UV light, this breakdown process is expected to occur only in the upper few centimetres of the water column.[24]

The numerous studies undertaken on the fate of TBT have indicated that it degrades by a stepwise debutylation mechanism to the less toxic DBT and MBT, which have also been detected in the aquatic environment.[8,35] Maureen and Willingham[67] have reported that the TBT degradation process may be explained as a sequential loss of an alkyl group from TBT to form non-toxic inorganic tin ultimately in the following manner: $R_3Sn^+ \rightarrow R_2Sn^{2+} \rightarrow RSn^{3+} \rightarrow Sn(IV)$. Complicating the issue of organotin persistence is the possibility of other degradation pathways for TBT species, including a number of possible redistribution reactions catalysed by environmental molecules such as amines, sulfides or other reactants. The possibility of environmental methylation of butyltins has been raised by recent reports of the presence of mixed butylmethyltin species in sediments, presumably arising by biological methylation of anthropogenic butyltin in the aquatic environment. A few of the possible reactions of Sn—C include the following:

$$2Bu_3Sn+ \rightarrow Bu_2Sn^{2+} + Bu_4Sn \quad (1)$$

$$Bu_2Sn^{2+} + Bu_3Sn^+ \rightarrow BuSn^{3+} + Bu_4Sn \quad (2)$$

$$Bu_3Sn^+ + Me^- \rightarrow Bu_3MeSn \quad (3)$$

At present, the source of the methyl moiety is unknown, but it may be due to redistribution and biogenesis of methyltin species.[68]

There are few reports on the biodegradation of TBT mediated by microorganisms viz bacteria, fungi, cyanobacteria and green algae in terrestrial and aquatic environment.[35,50,58,60,64] Barug[51] has reported that Gram negative bacteria, viz. *P. aeruginosa* and *A. faecalis*, and fungi, viz. *Tramatis versicolor* and *Chaetomium globosum*, could degrade TBTO via a dealkylation process. Pure cultures of the wood-rotting fungi, *Coniophora puteana* and *Coriolus versicolor* can also degrade this biocide to form OBT and MBT derivatives.[69] It is interesting to note that some *Pseudomonas* sp. have been reported to bioaccumulate TBT up to 2% of dry weight.[35,57] It has also been reported by Barug[51] that several other Gram negative bacteria possess the capability to accumulate TBTO without its breakdown. The high lipid-solubility of organotins ensures cell penetration and association with intracellular sites, and cell wall components also play an important role.[35] It is evident that the site of action of organotins may be both at the cytoplasmic membrane and at the intracellular level. Consequently, it is not known whether cell surface adsorption, accumulation within the cell, or both is a prerequisite for toxicity. TBT biosorption studies in fungi, cyanobacteria and microalgae indicate that cell surface binding alone occurred in these organisms, while studies on the effect of TBT on certain bacterial strains indicated that it can also interact with cytosolic enzymes.[45] The elimination of such hydrophobic compounds is facili-

tated by their biotransformation to water-soluble polar compounds. Thus, the metabolism of a compound generally reduces persistence, increases removal or elimination and results in a reduction of toxicity. Therefore, microbial degradation is probably the most predominant process for the breakdown of TBT in near-shore waters with DBT as the major degradation product.[70]

BIOCHEMICAL AND GENETIC BASIS OF TBT (ORGANOTIN) RESISTANCE IN BACTERIA

Genetic studies on TBT-resistant and -degrading bacterial strains from terrestrial and aquatic environments are limited with a few reports demonstrating the presence of plasmids but no correlation with TBT resistance.[26,33,71–73] In most cases, it has been demonstrated that the resistance-conferring genes are located on a chromosomal genome.[26,74] Fukagawa and Suzuki[72] reported the presence of genes conferring TBT resistance in *Alteromonas* sp. strain M1. They have successfully isolated, cloned and sequenced the gene that seems to be involved in the efflux of TBT employing a membrane-bound TBTCl-induced transport protein, possessing 108 amino acid residues encoded by an open reading frame of 324 nucleotides. This membrane protein has 48.5% of hydrophobic residues and shows more homology with transglycosylases of *E. coli* and other bacterial strains.[72] Therefore, this membrane protein has been predicted to be the most prominent resistance mechanism in this marine bacterial strain. Suzuki *et al.*[74] have further confirmed the taxonomic position of this strain by 16S rRNA sequencing and genomic sizing by pulse field gel electrophoresis using a contour clamped homogeneous electric field technique. These studies revealed that *Alteromonas* sp. M1 possesses a genome of 2240 kb. It is interesting to note that this strain is devoid of any plasmid, suggesting the exclusive presence of TBT-resistance genes on the chromosomal genome.[72]

FUTURE PROSPECTS FOR A MICROBIAL BIOREMEDIATION PROCESS

The application of natural microbial populations for bioremediation of organotin-contaminated sites is far away from a real large-scale field/commercial process, since little work has been done to explore the exact mechanism of biodegradation and the genes involved in the process. Therefore, we suggest that research is required to elucidate the basic mechanism of TBTCl bioaccumulation and biodegradation, employing molecular biologically and biochemical tools. These studies would ultimately enable use of natural and genetically modified bacterial strains for bioremediation of TBT-contaminated sites.

Acknowledgements

Dr S. K. Dubey is grateful to the Department of Ocean Development, India, for financial support for his grant proposal, "Studies on marine bacteria transforming organotins and development of microbial biosensors for monitoring organotins in marine environment". Mr Upal Roy also thanks the Department of Ocean Development, India, for financial support in the form of a junior and a senior research fellowship.

REFERENCES

1. Tisdale WH. *British Patent No.* 578 312 1943.
2. Evans CJ, Smith PJ. *J. Oil Colour Chem. Assoc.* 1975; **58**: 160.
3. Smith PJ. *Toxicological Data on Organotin Compounds*. International Tin Research Institute Publications, 538; 1978.
4. Smith PJ. *Structure Activity Relationships for Di- and Tri-organotin Compounds*. International Tin Research Institute Publications, 569; 1980.
5. Smith PJ. *Chemistry of Tin*, 2nd edition. Blackie Academic and Professional: London, 1998.
6. Alzieu CL, Sanjuan J, Deltreil JP, Borel M. *Mar. Pollut. Bull.* 1986; **17**: 494.
7. Alzieu CL, Sanjuan J, Michel P, Borel M, Dreno JP. *Mar. Pollut. Bull.* 1989; **20**: 22.
8. Dowson PH, Bubb JM, Lester JN. *Mar. Pollut. Bull.* 1993; **26**: 487.
9. De mora SJ, Stewart C. Phillips D. *Mar. Pollut. Bull.* 1995; **30**: 50.
10. De mora SJ. *Tributyltin – Case Study of an Environmental Contaminant*. Cambridge University Press: Cambridge, 1996.
11. Minchin D, Bauer B, Oehlmann J, Oehlmann US, Duggan CB. *Mar. Pollut. Bull.* 1997; **34**: 235.
12. Evans SM. *Biofouling* 1999; **14**(2): 117.
13. Champ MA. *Sci. Total Environ.* 2000; **258**: 21.
14. Champ MA. *Pollution prevention from ships and shipyards*. In *Proceedings of International Oceanology Symposium – 2001*, vol. I, Miami, FL, USA; 2001; 250.
15. Alzieu CL, Heral M. *Ecotoxicological effects of organotin compounds on oyster culture. In Ecotoxicological Testing for the Marine Environment*, (eds), Persoone G *et al.* vol. II, State University of Ghent, Ghent, and Institute of Marine Science Research, Bredence, Belgium; 1984; 187–196.
16. Laughlin RB, French W, Guard HE. *Environ. Sci. Technol.* 1986; **20**: 884.
17. Maguire RJ, Chau YK, Bengert GA, Hale EJ, Wong PTS, Kramar O. *Environ. Sci. Technol.* 1982; **16**: 698.
18. Maguire RJ, Tkaez RJ, Chau YK, Bengert GA, Wong PTS. *Chemosphere* 1986; **15**: 253.
19. Champ MA, Seligman PF. *Organotin: Environmental fate and effects*. Chapman and Hall: UK, 1997.
20. Hoch M. *Appl. Geochem.* 2001; **16**: 719.
21. Cleary JJ, Stebbing ARD. *Mar. Pollut. Bull.* 1987; **18**: 238.
22. Maguire RJ. *Environ. Sci. Technol.* 1984; **18**: 281.
23. Maguire RJ. *Water Qual. Res. J. Can.* 2000; **35**(4): 633.
24. Clark EA, Sterritt RM, Lester JN. *Environ. Sci. Technol.* 1988; **22**: 600.
25. Fukagawa T, Suzuki S, Fukunaga K, Suzuki T, Takama K. *FEMS Microbiol. Lett.* 1992; **93**: 83.
26. Suzuki S, Fukagawa T. *J. Ind. Microbiol.* 1995; **14**: 154.
27. Inoue H, Takimura O, Fuse H, Murakami K, Kamimura K, Yamaoka Y. *Appl. Environ. Microbiol.* 2000; **66**: 3492.
28. Boopathy R, Daniels L. *Appl. Environ. Microbiol.* 1991; **57**: 1189.
29. Atireklap SK, Tanabe S, Sanguansin J. *Mar. Pollut. Bull.* 1997; **34**: 894.
30. Craig PJ. Environmental chemistry of organotins. In *Comprehensive Organometallic Chemistry*, vol. **II**, Wilkinson G (ed.). Pergamon Press: Oxford, 1982; 979.
31. Hallas LE, Means JC, Cooney JJ. *Science* 1982; **215**: 1505.
32. Blunden SJ, Hobbs LA, Smith PJ. The environmental chemistry of

organotin compounds. In *Environmental Chemistry*, Bowen HJM (ed.). The Royal Society of Chemistry: London, 1984.
33. Singh K. Biocidal effect of organotin compounds. In *Frontiers in Applied Microbiology*, vol. **II**, Mukherjee KG, Singh VP, Garg KL (eds). Publication Print House: New Delhi, India, 1987; 297–316.
34. Pain A, Cooney JJ. *Arch. Environ. Contam. Toxicol.* 1998; **35**: 412.
35. Gadd GM. *Sci. Total Environ.* 2000; **258**: 119.
36. Cooney JJ, Wuertz S. *J. Ind. Microbiol.* 1989; **4**: 375.
37. Laurence OS, Cooney JJ, Gadd GM. *Microb. Ecol.* 1989; **17**: 275.
38. Cooney JJ. *Helgol. Meeresunters*. 1995; **49**: 663.
39. Yamada J, Tatsuguchi K, Watanabe T. *Agric. Biol. Chem. (Tokyo)* 1978; **42**: 1167.
40. Yamada J, Tatsuguchi K, Watanabe T. *Agric. Biol. Chem. (Tokyo)* 1979; **43**: 125.
41. Hallas LE, Cooney JJ. *Dev. Ind. Microbiol.* 1981; **22**: 529.
42. Hallas LE, Thayer JS, Cooney JJ. *Appl. Environ. Microbiol.* 1982; **44**: 193.
43. Pettibone GW, Cooney JJ. *J. Ind. Microbiol.* 1988; **2**: 373.
44. Cooney JJ, de Rome L, Laurence OS, Gadd GM. *J. Ind. Microbiol.* 1989; **4**: 279.
45. White JS, Tobin JM, Cooney JJ. *Can. J. Microbiol.* 1999; **45**: 541.
46. Skinner CE. In *Proceedings of Antifouling Symposium*, CIBA–GEIGY, Marienberg, West Germany 1971.
47. Christie AO. In *Proceedings of 3rd International Congress of Marine Corrosion and Fouling*, Gaithersburg, MD, USA; 1972; 676.
48. Mawatari S. In *Proceedings of 3rd International Congress of Marine Corrosion and Fouling*, Gaithersburg, MD, USA, 1972; 956.
49. Phillip AT. In *Australian OCCA Proceedings and News* (July): 1973; 17.
50. Barug D, Vonk JW. *Pestic. Sci.* 1980; **11**: 77.
51. Barug D. *Chemosphere* 1981; **10**: 1145.
52. Hallas LE, Cooney JJ. *Appl. Env. Microbiol.* 1981; **41**: 466.
53. McDonald L, Trevors JT. *Water Air Soil. Pollut.* 1988; **40**: 215.
54. Wuertz S, Miller CE, Pfister RM, Cooney JJ. *Appl. Environ. Microbiol.* 1991; **57**: 2783.
55. Suzuki S, Fukagawa T, Takama K. *Appl. Environ. Microbiol.* 1992; **58**: 3410.
56. Fukagawa T, Konno S, Takama K, Suzuki S. *J. Mar. Biotechnol.* 1994; **1**: 211.
57. Blair WR, Olson GJ, Brinckman FE, Iverson WP. *Microb. Ecol.* 1982; **8**: 241.
58. Cooney JJ. *J. Ind. Microbiol.* 1988; **3**: 195.
59. Blunden SJ, Chapman AH. *A Review of the Degradation of Organotin Compounds in the Environment and their Determination in Water*. International Tin Research Institute Publication, 626; 1983; 1–7.
60. Gadd GM. *FEMS Microbiol. Rev.* 1993; **11**: 297.
61. Dowson PH, Bubb JM, Lester JN. *Estuarine Coastal Shelf Sci.* 1996; **42**: 551.
62. Seligman PF, Valkris AO, Lee RF. *Environ. Sci. Technol.* 1986; **20**: 1229.
63. Sarradin PM, Lapaquellerie Y, Astruc A, Latouche C, Astruc M. *Sci. Total Environ.* 1995; **170**: 59.
64. Sheldon AW. In *Proceedings of 18th Annual Marine Coatings Conference*, Monterey, CA, USA 1978.
65. Stronkhors J, Hattum BV, Bowmer T. *Environ. Toxiol. Chem.* 1999; **18**: 2343.
66. Sheldon AW. *J. Paint. Technol.* 1975; **47**: 54.
67. Maureen EC, Willingham GL. *Biofouling* 1996; **10**: 239.
68. Matthias CL, Beluga JM, Olson GJ, Brinckman FE. *Environ. Sci. Technol.* 1986; **20**: 609.
69. Henshaw BG, Laidlaw RA, Orsler RJ, Carey JK, Savory JG. In *Record of the 1978 Annual Convention of B.W.P.A.*, Cambridge; 1978; 19.
70. Page SD. *Mar. Pollut. Bull.* 1989; **20**: 129.
71. Singh AP, Singh K. *Z. Naturforsch.* 1984; **39**: 293.
72. Fukagawa T, Suzuki S. *Biochem. Biophys. Res. Commun.* 1993; **194**: 733.
73. Miller CE, Wuertz S, Cooney JJ, Pfister RM. *J. Ind. Microbiol.* 1995; **14**: 337.
74. Suzuki S, Tsukamoto KK, Fukagawa T. *Microbios* 1994; **77**: 101.

