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Biodegradation of endosulfan by soil bacterial cultures

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ABSTRACT

Three bacterial isolates enriched from historically contaminated soil samples were investigated for their ability to use 100 mg L^{-1} of technical grade endosulfan as sole source of carbon and energy under aerobic conditions in liquid phase. Among the three isolates, maximum biodegradation ability was obtained by PT-3 which degraded >99% of 100 mg L^{-1} of endosulfan after 90 h of incubation and also used it as the source of sulfur. The isolate, PT-3 was identified as *Agrobacterium tumefaciens* and was able to use both α and β isomers of endosulfan with equal efficiencies without the accumulation of known toxic intermediates or end products. Endosulfan is known to be highly toxic to nitrogen fixing bacteria such as *Rhizobium* sp thereby reducing the fertility of soil. *A. tumefaciens* is a well known inhabitant of agricultural fields and therefore may be a valuable bioaugmenting agent for remediation of endosulfan contaminated sites.

1. Introduction

Pesticides play an important role in enhancing agricultural productivity by providing reliable control against harmful pests. However, many pesticides and their partially degraded products are highly persistent in the natural environment and cause undesirable effects on non-target lower and higher organisms. Among the various types of organochlorinated pesticides, a compound that is known to cause significant risk to higher organisms including human beings is endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodio-3-oxide). Endosulfan belongs to cyclodiene family of compounds and acts as a contact poison to wide variety of insects (Rani et al., 2003). Technical-grade endosulfan typically contains a mixture of stereoisomers, α-isomer and β-isomer in the range of 67–72% and 28–32%, respectively. Both isomers exhibit almost similar insecticidal activities but have significant differences with respect to their physicochemical properties (Schmidt et al., 2001).

Environmental risk assessment studies have demonstrated that exposure to endosulfan could result in both acute and chronic risks in non-target animals, mainly birds, fish and mammals (Sunderam et al., 1992). Fish in particular are very susceptible to endosulfan toxicity at a level of 1–20 ng L^{-1}. Human exposure to endosulfan can cause liver and kidney damage, nervous disorders, reproductive and developmental effects (ATSDR, 2000; Lu et al., 2000). Despite their negative impact on human health, India is one of the largest producers as well as the largest consumer of endosulfan in the world. Investigations by National Institute of Occupational Health (India) revealed that endosulfan was a causative factor in the incidences of serious health problems that impacted young and old human beings. Health effects including physical deformities, cerebral palsy, vision loss, and infertility were reported in the Kasargod district of Kerala where endosulfan was sprayed aerially on the cashew plantations for over two decades (NIOH, 2002). Serious environmental and health concerns have led to total ban on the usage of endosulfan in Kerala as well as intense search for post-application remediation methods.

Based on the foregoing discussion it is very explicit that the isomers of endosulfan are highly toxic to several life forms and therefore, it is important to develop technologies for the remediation of water containing concentrations of endosulfan that are much higher or just above permissible limits. Several methods have been used for the removal of endosulfan, and these include adsorption, chemical oxidation (or) reduction, filtration and biological degradation. However these techniques have their own limitations. For instance, adsorption and filtration involve mere phase transfer of pollutants without detoxification. Chemical oxidation of endosulfan leads to the formation of endosulfan sulfate, which is more persistent than the parent isomers and equally toxic (ATSDR, 2000). Biological treatment is considered to be a more feasible option in comparison to physicochemical methods because the latter involve high treatment costs and possibilities of

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causing secondary pollution problems. However, high concentrations of endosulfan often inhibit biological processes (Thangadurai and Suresh, 2013). Thus, employing specific physico-chemical or biological method for the treatment endosulfan contaminated matrices is not a feasible approach. An appropriate combination of physical, chemical and biological methods is required depending upon the concentration of endosulfan, nature of contaminated matrix and natural environmental conditions prevailing at the sites.

In a previous publication, we have reported reductive degradation of endosulfan to completely dechlorinated and desulfurized hydrocarbon end product using magnesium–palladium bimetallic system (Thangadurai and Suresh, 2013). However, high cost of palladium and requirement of an agent (such as organic solvent or detergent) to solubilize endosulfan were the major shortcomings and therefore we examined alternate remediation options. Retrospection of literature revealed that a wide range of microorganisms, mostly bacteria, few fungi and cyanobacteria are capable of utilizing endosulfan as a sole source of carbon and/or sulfur (Sutherland et al., 2002a,b; Sethunathan et al., 2004; Kwon et al., 2005; Lee et al., 2006; Weir et al., 2006; Kumar et al., 2007, 2008; Goswami et al., 2009; Li et al., 2009; Kataoka et al., 2011; Singh and Singh, 2011; Verma et al., 2011; Giri and Rai, 2012). Also, endosulfan degradation was reported to be more effective through the use of consortium of bacterial strains in comparison to individual bacterial isolates (Kumar and Philip, 2006; Rama and Philip, 2011). Fig. 1 illustrates the key pathways involved in the biotransformation of endosulfan by microorganisms with associated enzymes.

Biological remediation of high concentrations of endosulfan recovered from contaminated water through physical processes such as adsorption or filtration may be a promising approach. Although a multitude of bacteria have been shown in laboratory assays to have the potential to biodegrade endosulfan, rarely have the populations that are capable of metabolizing endosulfan at high concentrations (>100 mg L^{-1}) in aqueous phase been reported. Therefore it is crucial to identify microbial strain(s) that are capable of degrading high concentrations of both α- and β-isomers of endosulfan without the accumulation of toxic metabolites in treated water. In the current investigations we report the ability of microbial isolate(s) to degrade/transform higher concentrations of endosulfan without the accumulation of toxic metabolites and end products. The main objectives of the present investigations were to: (a) Isolate, screen and identify microorganisms for their ability to biotransform high concentrations of endosulfan. (b) To demonstrate the ability of pure microbial isolate(s) to utilize endosulfan as a source of carbon and sulfur.

2. Materials and methods

2.1. Chemicals and growth media

Technical grade endosulfan (consisting of α and β isomers in the ratio of 2:1), pure α-endosulfan and β-endosulfan were purchased from Sigma–Aldrich, St. Louis, Missouri, USA. The purity of α and β endosulfan isomers was 99%. Tween-80 (nonionic surfactant),

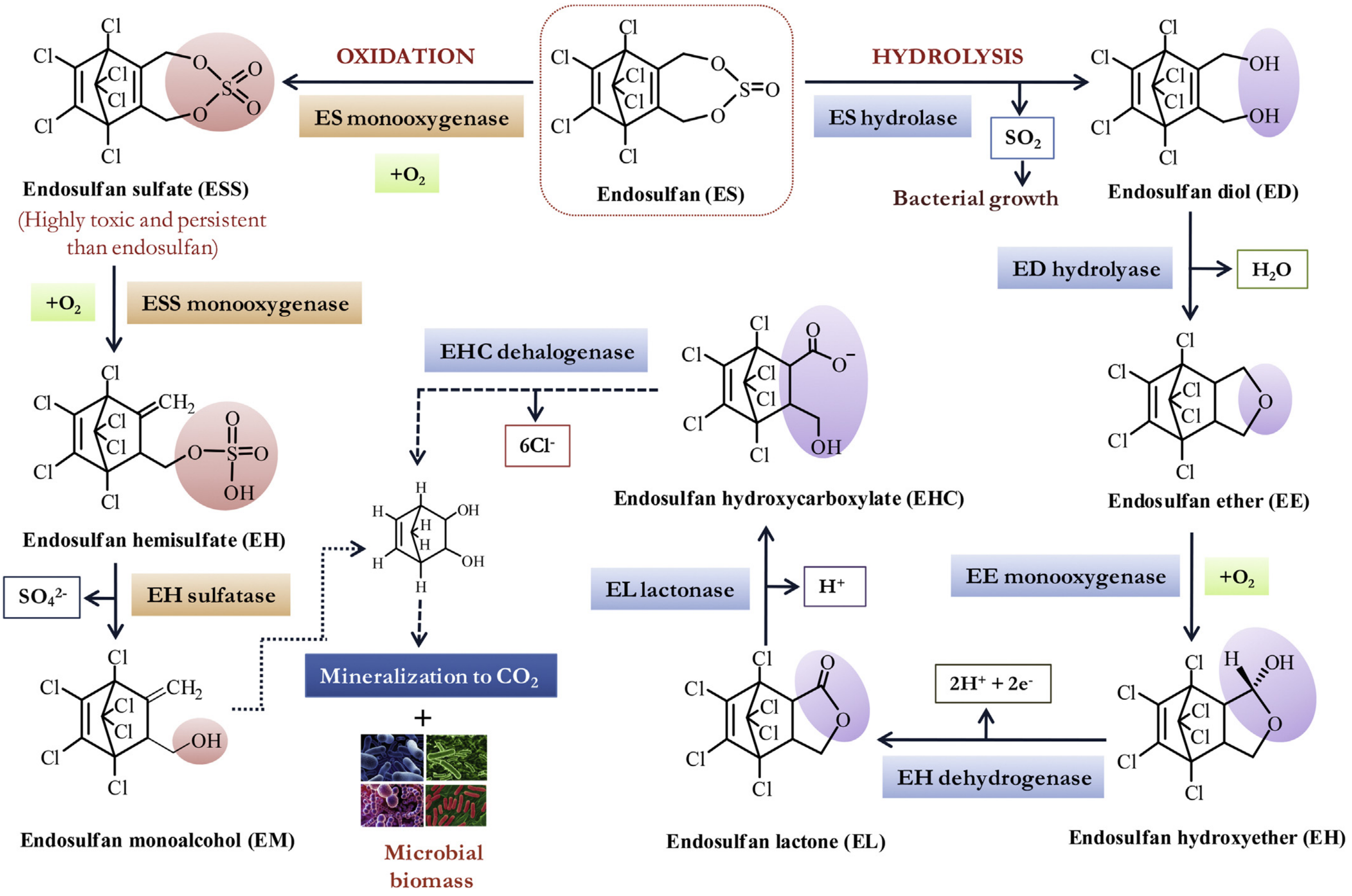

Fig. 1. Pathways involved in the biotransformation of endosulfan by microorganisms and associated enzymes (Kwon et al., 2005; Weir et al., 2006; Kumar et al., 2007; Li et al., 2009; Kataoka et al., 2011; Verma et al., 2011).