BIOREMEDIATION OF ENDOSULFAN

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Introduction

Endosulfan has proved to be a cheap and efficacious insecticide. It provides ongoing value in the protection of conventional and transgenic cotton crops against Heliothis and other insect pests. However, endosulfan has been associated in the past with off-site residue problems that threaten its ongoing registration. There are two primary areas of concern in this regard. The first of these is with respect to environmental impacts of endosulfan contamination of downstream waterways. The second centres on concerns regarding endosulfan residues identified in locally grown beef through consumption of contamination of pastures. The cotton industry has responded to the concerns surrounding endosulfan usage by developing and achieving widespread adoption of Best Management Practices for Minimising the Impact of Pesticides. Whilst these practices should reduce the risk, the seriousness of the concern with respect to the two issues identified above requires development of mechanisms to directly address contamination. Many of the problems with endosulfan residues would be avoided if water at risk could be quickly decontaminated. This could be achieved by on-farm bioremediation of tail water with specialised enzymes (proteins that catalyse chemical reactions). This paper describes early progress in a CRDC and CRC supported CSIRO project that is working towards this goal. A more detailed description of this work will appear in Sutherland *et al.*, 2000a,b (1,2).

Bioremediation (detoxification using biological materials) is possibly the simplest method for the treatment of large contaminated sites (3). Bioremediation using live microorganisms requires sources of nutrients specific to the requirements of the remediating microbe. Whilst the environment of the contaminated site may provides these nutrients, generally supplementation with additional nutrients is required. Bioremediation with enzymes does not require nutritional supplementation and holds potential in environments with low nutrient levels, ie. Waste water. Whilst enzymes have not yet been commercialised for bioremediation of insecticides, they have found increasing use over the last decade in a wide range of industrial and domestic applications. For example, protease enzymes are widely used in laundry and cleaning products.

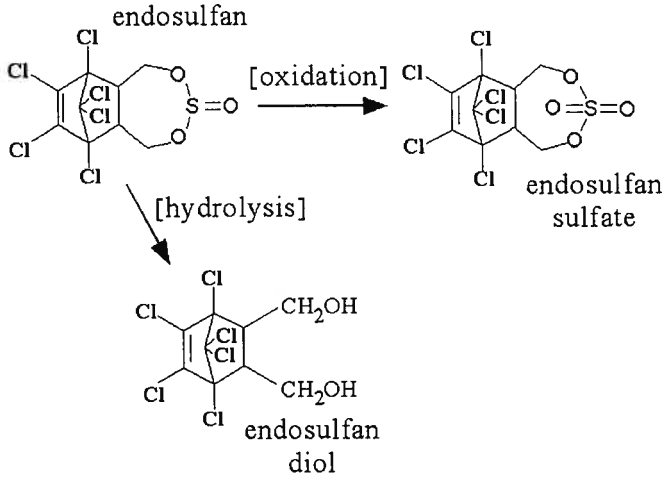
CSIRO Entomology is developing an enzymatic bioremediation technology for detoxifying endosulfan and related residues in contaminated water prior to its release from the farm and into the waterways. The work benefits from being part of a larger CSIRO Entomology project to develop bioremediation enzymes for a range of pesticides. Products of this larger project, which has already developed enzymes for organophosphates and pyrethroids, are licensed to Orica Australia Ltd (formerly ICI Australia). Orica Australia Pty Ltd has a large watercare Division and extensive marketing and distribution network in rural Australia.

Sources of Bioremediating Enzymes

It is well established that contact with contaminants leads to adaptation of indigenous soil microbial populations (4) and the most common sources of enzymes with bioremediation potential have so far been pesticide tolerant soil microorganisms. Most pesticides are not particularly toxic to bacteria but are worthwhile nutrient sources to microbes if they are recurrently available in more than trace amounts. This is frequently the case where relatively persistent pesticides are deliberately applied to soil for treatment of soil pests or where accidental/incidental exposures occur regularly.

Numerous studies have described the degradation of endosulfan in soils or by soil microorganisms (5-15). The insecticide is degraded by attack at the sulfite group via both oxidation and hydrolysis to form the toxic endosulfan sulfate and the non-toxic endosulfan diol, respectively (Figure 1).

Figure 1. Most common products of endosulfan degradation.



The formation of endosulfan sulfate occurs only through biological transformation, whereas hydrolysis to the diol occurs readily at alkaline pH (11). Many previous studies describing degradation of endosulfan in microbial cultures were unable to calculate the contribution of chemical, as compared to biological, hydrolysis. This is because microbial growth often led to an increase in pH of the culture medium to a point at which chemical hydrolysis became significant. Furthermore, losses of endosulfan from culture media or soils can occur readily through both volatilisation and adsorption to surfaces (16). This requires care in the culture of the organisms and detection of product formation to ensure that losses of the insecticide are the result of biological degradation.

Isolation of an Endosulfan-Degrading Bacterium

We investigated soil with a history of exposure to endosulfan as a potential source of endosulfan-degrading bacteria, using a rigorously controlled experimental system to minimise losses of endosulfan due to chemical hydrolysis, volatilisation and adsorption. Enrichment of a culture of soil bacteria capable of degrading endosulfan was achieved and maintained by providing endosulfan as the only sulfur source. Endosulfan is a poor biological energy source and previous attempts to enrich for endosulfan-degrading microorganisms, using the insecticide as a carbon source, have been unsuccessful (5,17). However, endosulfan has a relatively reactive cyclic sulfite diester group (18, Fig 1) and in our experiment microorganisms were selected for their ability to release the sulfite group from endosulfan and to use this as a source of sulfur for growth. Since the removal of the sulfur moiety dramatically decreases vertebrate toxicity (19,20), this method selects organisms capable of detoxifying the insecticide.

After successive sub-culturing with endosulfan as the only source of sulfur, analysis of the soil culture confirmed substantial disappearance of endosulfan, with a simultaneous increase in bacterial mass (Figure 2). Commercial endosulfan contains a mixture of two diastereoisomers: *alpha*-endosulfan and *beta*-endosulfan, in a ratio of 7:3 respectively. Addition of *alpha*-, *beta*- or technical grade endosulfan, or a range of other sources of sulfur, promoted growth of the culture to varying degrees. No growth was observed in the medium without the addition of a sulfur source. Rates of endosulfan metabolism increased with each successive subculturing and a pure culture was obtained after six months. Growth of the final culture on solid media gave rise to slow growing translucent colonies that became easily visible after 3-4 days and reached 3 mm diameter after 6 days. Broth cultures of individual colonies degraded endosulfan by both hydrolytic and oxidative pathways. This isolate was named strain ESD (Endosulfan Degrading).

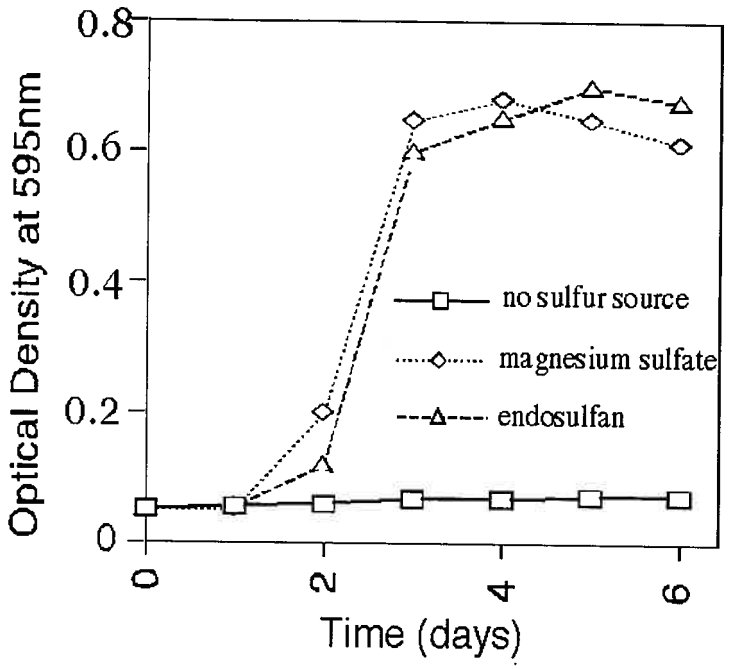


Figure 2. Growth of strain ESD in minimal media with 50uM magnesium sulfate, technical grade endosulfan or no sulfur source.

Characterisation of Endosulfan-Degrading Bacterium

Analysis of the formation and decay of endosulfan and its metabolites in growing cultures led us to propose a pathway of endosulfan metabolism by strain ESD (Figure 3). According to this pathway the insecticide is either oxidised or hydrolysed. The oxidation reaction is favoured for the *alpha* isomer and produces endosulfan sulfate. Preferential oxidation of this isomer has been reported previously and it is thought that it contributes the majority of endosulfan sulfate found in the environment (12, 21, 22). The hydrolytic pathway of degradation progresses through a novel metabolite, which has molecular characteristics predictive of endosulfan monoaldehyde (Figure 3). Oxidative cyclisation of the mono-aldehyde then leads to endosulfan hydroxyether, which is further metabolised to polar products. This pathway is substantially different from the degradation pathway described in other studies (8, 12, 22, 23). The pathway proposed in these other studies involves a double hydration to produce endodiol followed by a dehydration to produce endosulfan ether.

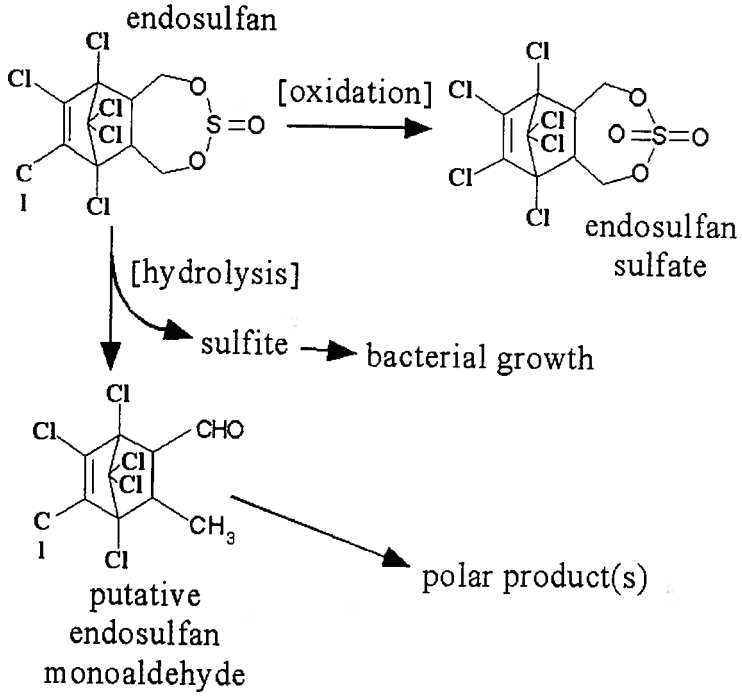


Figure 3. Pathway of endosulfan metabolism proposed for strain ESD.

As the novel putative monoaldehyde product has not been described as a product of chemical degradation, we are confident that the degradation we observe by strain ESD is biological. Currently, the culture metabolises 50 μM endosulfan to undetectable levels in less than 4 days. This rate is significantly higher than those measured in previous studies, in which both biological and chemical degradation often contributed to rates of endosulfan disappearance. Our study differs from previous studies by the application of strong selection pressure on the culture to release the sulfur moiety from the insecticide, allowing us to enrich for the degradative activity and concurrently detoxify the insecticide. We are currently characterising the hydrolytic ability of this culture as a potential enzymatic bioremediating agent for endosulfan.

Acknowledgments

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