BIOREMEDIATION – WHAT CAN WE DO?

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Introduction

Bioremediation can be defined as any process where biological agents provide a practical solution to the unwanted presence of a chemical substance. In providing this solution, the risk or probability that a hazard may act must be substantially reduced. Thus, in its broadest sense, even the introduction of GM technology to reduce dependence on insecticides (Fitt, 2000), or the encouragement of beneficial predators of pests could be included in bioremediation.

However, in this paper the term will be restricted to processes where a more specific biological agent, usually a microbe or microbial product or a plant is recognised as reducing the risk from agrochemicals such as insecticides or herbicides. In particular, attention will be directed towards those processes acting to reduce the "half- life" of the chemical as a contaminant in the local environment. The half life is the time taken for the amount of chemical present to be reduced to half a baseline value. The term remains very broad though, since a range of physical or chemical actions mediated by these biological agents could be involved in reducing the amount of the chemical present.

The Cotton CRC and the CRDC have both funded our research in this area for years, supporting several field researchers including Francisco (Paco) Sanchez-Bayo, Angus Crossan, Michael Rose and in CSIRO. It is time to consider how the outputs of this research could be used to the benefit of cotton farming. These possible benefits will be considered in the context of several areas currently under research to reduce the risk from chemicals such as:

- Biologically active areas on farms including filtration and sedimentation zones

- Constructed wetlands; considering the ubiquitous distribution of biota when water is in ample supply, the complete system for circulation of water on cotton farms with its associated biota can be considered as having a role in bioremediation.

- Gin trash disposal using composting; there are opportunities for risk reduction.

- Bioremediation enzymes; these products being researched by CSIRO Entomology provide opportunities for specific purposes.

Natural bioremediation on farms

It is important to realise that nature already provides many examples of bioremediation. For most substances, a problem for some may be an opportunity for others. The main concern regarding toxic agrochemicals is their ecotoxic effects on biota. Some organisms can benefit from the presence of chemical substances that are toxic to others as a result of the adaptation of their metabolism so that the agrochemical may become a growth substrate. This is recognised with chemicals such as 2,4-D and the triazines such as atrazine and prometryn. Some organisms can use these substances to provide energy for their growth, degrading their ring structures with special enzymes and using the simpler breakdown products for normal metabolism.

The 2005 ACGRA Grower of the Year, Neek Morawitz, has designed a tailwater return system allowing several beneficial processes (see the Cotton CRC's 2006 brochure *Design principles for healthy waterways on cotton farms).* Using a vegetated tail-drain system broadening into a central sump, with a vegetated perimeter, tailwater is freed of sediments by the slower flow velocity in the sump and biofilms on plant stems and other surfaces may strip pesticides from the water phase or deposit them in sediments. The cleaned-up water can then be returned to the main 2000 ML reservoir.

Recent research (Rose et al., 2006) has shown that rates of degradation of several pesticides including endosulfan, aldicarb, prometryn and diuron was actually faster in water obtained from farm storages than water sampled from a constructed wetland. Of the five pesticides examined, only chlopyrifos degraded faster in water from the wetland. Suggested reasons for the observation include a greater number of pesticide-degrading microorganisms in the storage dam waters, which are more frequently exposed to cotton pesticides; and/or the presence of more organic matter in the wetland samples, which can stabilise contaminants, limiting their volatilisation and hydrolysis. Exposure to light significantly reduced the rate of endosulfan removal, whereas light significantly increased the rate of chlorpyrifos removal. This research emphasises the importance of considering each pesticide in context, but also not to neglect the positive effects possible in standard water recirculation systems.

Constructed wetlands

A pilot-scale, ponded wetland consisting of an open pond and a vegetated pond in series (see Fig. 1) was constructed on a cotton farm in northern New South Wales, Australia, and assessed for its potential to remove pesticides from irrigation tailwater (Rose et al., 2006). Ten incubation periods ranging from 7-13 days each were conducted over two cotton growing seasons to monitor removal of residues of four pesticides applied to the crop. Reductions in residues ranging 22-53% and 32-90% were observed in the first and second seasons respectively. Average half-lives during this first season were calculated as 21.3 days for diuron, 25.4 days for fluometuron and 26.4 days for aldicarb over the entire wetland. During the second season of monitoring, pesticide half-lives were significantly reduced, with fluometuron exhibiting a half-life of 13.8 days, aldicarb 6.2 days and endosulfan 7.5 days in the open pond. Further significant reductions were observed in the vegetated pond and also following an algal bloom in the open pond, as a result of which aldicarb and endosulfan were no longer quantifiable. Partitioning onto sediment was found to be a considerable sink for the insecticide endosulfan. These results demonstrate that macrophytes and algae can reduce the persistence of pesticides in on-farm water and provide data for modelling. In future it may be possible to enhance breakdown in wetlands of particular pesticides such as diuron or prometryn, for which microbes capable of their degradation have been isolated in laboratory studies (Rose, 2006)

Figure 1. Constructed wetland with non-vegetated settling pond followed by vegetated pond

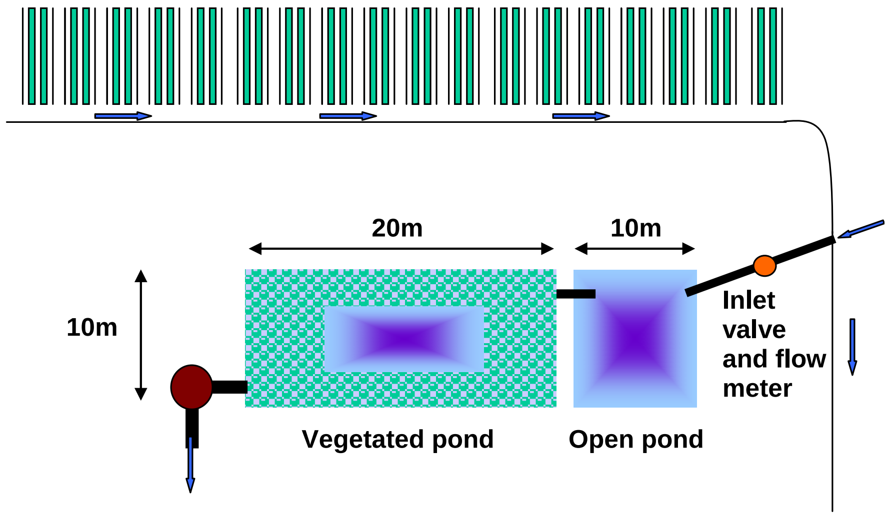
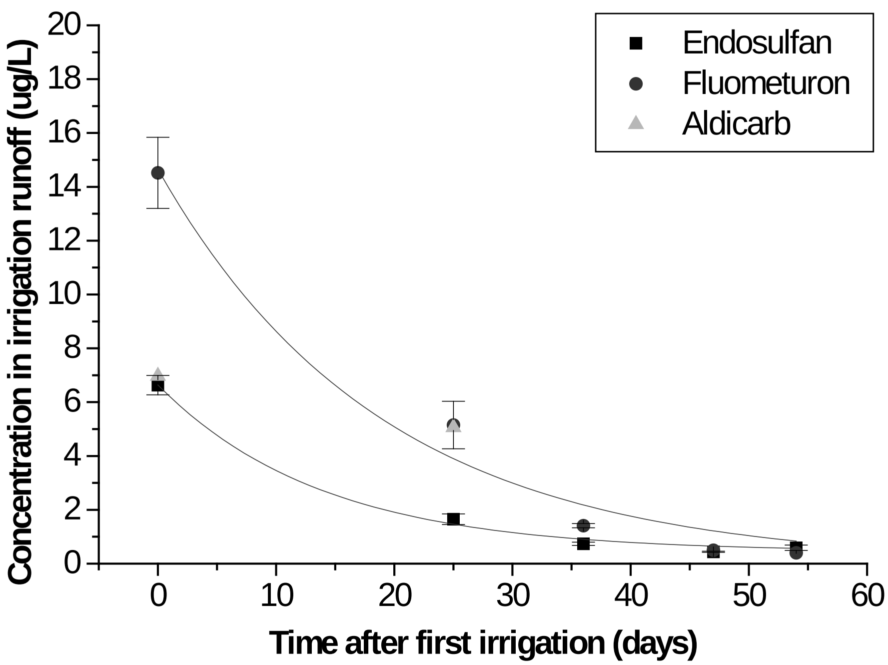


Figure 2. Declining concentration of pesticides in runoff as a result of remediation processes



Turning the problem of gin trash into a solution

Our research has shown that cotton gin trash should not be assessed as hazardous waste (Crossan and Kennedy, 2005). Properly managed, such as by composting, it is only slightly more risky than solid waste. This favourable assessment has possibly saved the cotton ginning industry $1.2billion dollars, discounted over the next 20 years (see www.crdc.gov.au/documents/HASSALL%20Cotton%20Trash%20Classification.pdf). Currently, we are testing the hypothesis that gin trash could be incorporated into surface soil to trap pesticides from runoff water and also to assist in their bioremediation by microbes.

Bioremediation enzymes

There is interest in the use of formulated enzymes rather than live bacteria as bioremediation agents, a process known as enzymatic bioremediation. This approach uses cloning biotechnology in bacteria to provide bioremediation enzymes as dried powders able to be directly applied in the field. The method has the advantage that the power of genetic engineering can be used in the laboratory to maximize enzyme potential using the various techniques of *in vitro* enzyme evolution now available, yet the final product does not involve the release of a genetically engineered organism. Enzymatic bioremediation is particularly suited to situations where rapid remediation is needed: at most a few hours, perhaps as little as a few minutes.

Research conducted by the group led by Dr John Oakeshott and Dr Robyn Russell at CSIRO Entomology in collaboration with Orica Australia to produce bioremediation enzymes gives another option for the cotton industry to consider as a means of risk reduction from pesticides. The development of enzymes for remediation (Sutherland et al., 2003) generally involves three stages: (i) the identification of a template enzyme from natural sources; (ii) isolation of the gene encoding the enzyme; and (iii) improvement of enzymatic properties in the laboratory. The most common sources of enzymes with bioremediation potential have so far been pesticide-tolerant soil microorganisms. The source bacteria generally have adapted to growth on contaminated soils, having the ability to detoxify pesticides or the ability to use the pesticide as a worthwhile nutrient source.

Enzymatic bioremediation is potentially a rapid method of removing environmental pesticide residues (Sutherland et al., 2003). Applications include the treatment of residues resulting from agricultural production and processing industries, such as the treatment of irrigation waters, surface-contaminated fruit and vegetables and spent dip liquors. A current specific application for some organophosphate degrading enzymes involves detoxification of stockpiles of chemicals. Effective and affordable remediation requires highly specialized enzymes, so protein engineering techniques are being used to improve properties of various source enzymes to enhance catalytic rates, stability and substrate range.

Trials with an optimized organophosphate-degrading enzyme have shown the feasibility of such technology in various applications, including a successful trial to remediate organophosphate in cotton tailwaters. The enzymes developed for environmental remediation for specific pesticide classes also have applications as antidotes for high-dose pesticide poisonings and as prophylaxis for people at risk of high pesticide doses.

Current status of the bioremediation enzyme research

Enzymes as usable products arising from this research are currently available for OPs, carbamates and pyrethroid insecticides. Unfortunately, one form of a cloned enzyme able to successfully degrade endosulfan requires expensive cofactors too impractical to prepare an effective product for application in the field. A project is currently under way on the production of bioremediation enzymes for diuron and its toxic breakdown product (metabolite). The Cotton CRC is involved in this research.

It has been suggested in a milestone report (Cotton CRC project 2.3.04, January 31 2006) that a pro-active approach to the availability of management tools for reducing chemical risk might be beneficial in prolonging the lifetime of chemicals such as diuron when their registration is at risk. Diuron is one such chemical currently under review and proposed restriction of its use by the APVMA.

The importance of monitoring

The positive value of monitoring pesticide levels in water has been demonstrated by the public response to the North-West Regions Water Quality Program conducted by the Department of land and Water Conservation during the 1990s. This has shown a sharp fall in the extent of river contamination for chemicals such as endosulfan during the period in which the BMP and GM technology were introduced to the cotton industry (Muschal, 2003), validating the effectiveness of these measures as environmental solutions.

Equally, it would be important to monitor water quality on farms so that cotton farmers have feedback about the degree of hazard pesticide residues present and also how their management can reduce their risk. Simple 'dip-stick' test kits for water quality could be made available with some investment in research and development, an area to which the cotton industry has already made a significant contribution.

Conclusion

This short review has provided information on several possible actions regarding bioremediation, which could all be undertaken to reduce risk from agrochemicals. The benefits possible from managing and enhancing bioremediation include:

- Reduced risk to the riverine ecosystems
- Promotion of ecosystem services related to biota and wildlife such as birds, fish and even aquaculture
- Improved water quality for wildlife and livestock
- An improved image of cotton farming

There would be some research costs involved in gaining these benefits but much of the research required has already been done. The paper will discuss how these environmental benefits might be achieved with minimum cost to cotton farmers.

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