PAPERS PRESENTED

Bioremediation in the Great Barrier Reef Marine Park

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This morning I would like to welcome you to the Great Barrier Reef Marine Park Authority workshop on bioremediation. This workshop is part of the Marine Park Authority's role as Scientific Support Coordinator in the oil spill contingency plan arrangements for the Great Barrier Reef Region.

What is bioremediation?

In general this means a biological process which improves or remedies a situation. In the context of oil spills, bioremediation would include any biological process which mitigates the effects of an oil spill. Recently bioremediati'on has come to mean the application of oil consuming bacteria or the use of fertilisers to promote bacterial growth, to enhance the natural degradation of oil.

Oil in the oceans

The treatment of spilled oil in the ocean by one means or another needs to be put into the context of oil which occurs naturally and that which is introduced by human activity. From 1974 to 1980 it was estimated that 42% of the total amount of petroleum entering the worlds oceans was the result of either shipping operations or casualties. This is out of a total world-wide input of oil into the sea of some 3.2 million tpnnes per annum.

Between 1981 and 1989, there appears to have been a decrease in accidental spillage of oil into the sea. The accounts of major recent oil spills, the Kharg V, Exxon Valdez, the Mega Borg and the recent Kuwait oil pouring into the Arabian Gulf have highlighted the need for more effective means to combat large unexpected amounts of oil.

The Great Barrier Reef Marine Park

'In the Great Barrier Reef Marine Park, while we have 'no drilling which could lead to an oil spillage, we do have shipping and vessel operations which could cause a significant oil spill.

The Great Barrier Reef Marine Park contains many natural hazards. There are approximately 2900 reefs, 300 coral cays, 600 high islands, many submerged shoals and reefs, narrow shipping lanes, strong trade winds, occasional cyclones and localised strong currents. The shipping route is circuitous and wedged between reefs and the' mainland coast, particularly north of Cairns and through Hydrographers Passage. In the southern Great Barrier Reef the width and depth of the, shipping channel increase significantly.

Traffic in the Great Barrier Reef Marine Park

The volume of traffic through the Great Barrier Reef Region is quite substantial by Australian standards. Some 2000 vessels travel through the Great Barrier Reef Region per annum, of which 200 are tankers. Since 1970, for vessels over about 24 metres, there have been about 175 recorded "incidents" in the Great Barrier Reef Region. These "incidents" include collisions, groundings, near misses etc. Most of these recorded incidents have occurred in ports and about only six of these have involved pollution. The major incident, of course, of which people are aware was the grounding of the 'Oceanic Grandeur" in 1970 in Torres Strait, in which it was estimated that 1400 to 4000 tonnes of oil were spilt.

Cargoes carried through the Marine Park include bauxite from Weipa to Gladstone in bulk carriers of up to 70 000 dead weight tonnes, coal in vessels up to 140 000 dead weight tonnes, refined product in carriers of 25 000 to 30 000 dead weight tonnes and crude oil and fuel oils in tankers of up to 100 000 dead weight tonnes. The maximum vessel size is approximately 140 000 dead weight tonnes.

Protection of the Great Barrier Reef Marine Park

In 1987 in recognition of the uniqueness of the Great Barrier Reef and the difficulties encountered by reef shipping, the International Maritime Organisation passed a resolution recommending pilotage for vessels over 100 metres and all loaded tankers. Since then, about 90% of vessels passing through the Reef Region have been piloted and only about ten tankers per annum are currently unpiloted. Although compliance with the voluntary resolution regarding pilotage was good, the Australian Government felt that it was not sufficient protection for the Reef and persuaded the International Maritime Organisation in late 1991 to pass resolutions declaring the-Great Barrier Reef Region a "Particularly Sensitive Area", the first in the world, and further resolution supporting moves by the Australian Government to make pilotage compulsory in the northern Great Barrier Reef and Hydrographers Passage for all vessels over 70m and all loaded tankers. Legislative changes are proposed to the Great Barrier Reef Marine Park Act to introduce this measure in October 1991.

National significance of the Great Barrier Reef Marine Park

The importance of the Great Barrier Reef is recognised internationally; it was inscribed on the World Heritage List in 1981, the International Maritime Organisation has designated the Capricornia Section of the Marine Park as 'An Area to be Avoided' and more recently the whole Great Barrier Reef as the worlds first Particularly Sensitive Area. The Great Barrier Reef Marine Park zoning scheme meets the World Conservation Union (IUCN) categories for protected areas. As well it meets the criteria for Marine Biosphere Reserves although is has not been declared as such.

Economic significance of the Great Barrier Reef Marine Park

As well as having enormous natural significance, the Great Barrier Reef has a significant economic importance. The value of tourism is believed to be growing by about 10% per annum. In 1990 it was estimated that some 2.5 million visitor trips were made to the Great Barrier Reef Region including the adjacent mainland. Tourism is

estimated to generate some $400-500 million per annum. There are 21 resorts and an estimated 300 charter boats servicing the tourist industry.

As, well as tourism, fishing is a major commercial activity in the Great Barrier Reef Region.:' Together, commercial'and recreational fishermen are estimated to generate , some $400 million per annum. The main commercial fishery is trawling, which occurs in the. vicinity of and adjacent to the shipping channel. The major recreational fishery is reef fishing which occurs mainly from small speed boats.

REEFPLAN

The Great Barrier Reef Marine Park Authority, concerned about the possibility of an oil spill in the Great Barrier Reef Region, asked the then Federal Department of Transport in the early 1980s to assist in the development of an oil spill contingency plan for the Great Barrier Reef. In 1987 REEFPLAN came into effect, as a supplement to the National Plan to Combat Pollution of the Sea by Oil. The objectives of REEFPLAN are:

1. to provide guidance for pollution response;
2. to provide guidance for planning;
3. to provide guidance for intergovernmental cooperation in the response.

The Great Barrier Reef Marine Park Authority sits on the Queensland State Oil Pollution Committee and GBRMPA coordinates environmental advice to the Committee. State and Federal authorities and non government organisations also sit on the Committee and cooperate in response arrangements. In the event of an actual spill, the On Scene Coordinator (OSC), appointed by the State Government, has overall charge of the operations and the clean-up of an oil spill. The OSC is advised by the State Committee and all response participants report to the OSC. The Marine Park Authority, as well as sitting on the State Committee, has the responsibility for coordination of scientific and environmental advice reporting directly to the OSC. The Marine Park Authority also has the responsibility, with the State Committee, for the appointment of a Media Liaison Officer to coordinate media interactions.

Under the Scientific Support Coordinationresponsibility, the Authority has responsibility for accessing the scientific database, advising the OSC regarding clean-up actions and sensitive sites in the event of a spill, and monitoring the effects of a spill for economic and environmental consequences. Under this role, the activities that the Authority has undertaken include the conduct of workshops for Scientific Support Coordinators, the conduct of workshops on specific topics for SSCs and the development of a pilot coastal resource atlas on a user friendly computer system. This has now been taken over by the State Committee and is funded through the Australian Maritime Safety Authority for the development of a Queensland-wide resources atlas and small oil spill model. The Marine Park Authority has also funded research into the effects of oil and dispersant on corals.

Under REEFPLAN, response equipment is available in major ports along the coast with particular concentrations of equipment in Townsville and Brisbane. As well there are national and international arrangements in which the Australian Maritime Safety Authority participates. Those arrangements have been further developed following the Exxon Valdez spill and recognition of the necessity for using internationally held

equipment. Local equipment includes booms, skimmers, stock piles of dispersant, spray pumps, radios, dinghies etc. However for a total Queensland coastline in the Great Barrier Reef Marine Park of some 2000km, there is less than 5km of boom available.

REEFPLAN, with support from the National Plan, is designed to cope with spills of up to about 1000 tonnes. One effect of the Exxon Valdez spill has been to indicate to governments and other people that a response is required for spills greater than 1000 tonnes. As a result moves are underway to improve Australia's spill response capability to 10 000 tonnes. This is largely being developed through funding from the shipping industry, and the Australian Institute of Petroleum establishing a major stockpile of equipment in Victoria available to be deployed anywhere around the country in the event of a major spill.

Difficulties in the Great Barrier Reef Marine Park

Within the Great Barrier Reef Marine Park, the major difficulties include distance of reefs and islands from the shore, the fact that much of the year the weather is inhospitable, the fact that significant amounts of the Great Barrier Reef Marine Park are in very remote areas and the fact that the adjacent population is relatively small, in the vicinity of 300 000. In the event of a large spill, or any remote spill, response effectiveness is likely to be very limited. Taking these factors into account, it is unlikely that much more than some defensive booming, shoreline clean-up and natural weathering could be undertaken in the event of a big spill. It is clear that while small accessible spills may be able to be combated, large remote spills will be almost impossible to combat. Impacts are most likely to occur on islands, the mainland coast, fringing reefs, mangroves and seagrasses. Offshore reefs may be unaffected, or at least relatively so, as oil may not strand on these.

Given the length of coastline and the number of reefs in the area under consideration, in association with the resources available to a country like Australia, it would seem that, given some response capability, a focus on prevention is probably better than a focus on cure. In this respect the achievement of compulsory pilotage and consideration of other measures such as double hulled vessels and improvement of navigation aids may be a wiser investment of funds, particularly given developing Australian and existing international arrangements. Education of vessel masters and users of the Marine Park is also likely to be of benefit.

Bioremediation

Given the difficulties outlined above, some consideration of the alternatives available for clean-up in the event of a spill is required. Currently the major alternatives are natural weathering, application of dispersant, skimming and salvage of oil, and more recently, in situ burning and bioremediation. Natural weathering (a form of bioremediation) may often be the most environmentally acceptable method of treatment. However this may not be acceptable, particularly if mangroves are about to be threatened, or if the spill is large, or heading for sensitive or valuable areas. The application of dispersant is always a difficult issue. Dispersants are themselves toxic and in some cases it is possible that the toxicity of the dispersant/oil mixture is higher than the toxicity of the dispersant or the oil alone. Studies to date are tending to show in tropical environments that dispersed oil is more toxic than oil to coral and to

seagrasses in terms of recovery, but that dispersed oil leads to a shorter recovery time than straight oil in the case of mangroves. The philosophy on the use of dispersants in the Great Barrier Reef Region is that they are not to be used in the immediate vicinity of coral or seagrass beds: but in open water situations dispersant may be preferable. Dispersant and in situ burning are the only two methods known, to be able make significant difference to the volume of oil which has been spilled. Physical recovery of oil can at 'most pick up some 30% of spilled oil. Dispersant may affect 60 - 70% of spilled oil. The relatively new technique of in situ burning was attempted in; a trial study for the Exxon Valdez spill and proved to be remarkably successful with some 95% of the contained oil being burnt. More testing of this method is required, however it is still necessary to contain oil before burning can be effective.

A promising development is bioremediation. It is a relatively new method that was tested extensively during the Exxon Valdez spill. It involves the application of bacteria and/or the application of fertilisers to stimulate bacterial production. Before it is applied within the Great Barrier Reef area there is a need to investigate some of the potential problems. Do bacteria need to be introduced? If so, are they acceptable in the Great Barrier Reef Marine Park? Is the addition of nutrients in the Great Barrier Reef Marine Park an acceptable activity given the concern expressed by the Marine Park Authority and others about nutrient inputs from the mainland through agriculture? What is the effectiveness of bioremediation? Although it has been tried in cold temperate waters and arctic waters, what is its effectiveness in warm conditions? Is it effective in open sea situations or is it only effective in beach situations? Is it effective in coral 'reef situations, sandy beach and rocky shore situations? What are the costs and benefits of bioremediation? These are the questions in which the Marine Park Authority is interested in relation to the application of this technique as a tool for oil spill clean-up. This workshop has been designed to address some of these questions.

Workshop objectives

The objectives of this workshop are:

1. to summarise the existing information on bioremediation;
2. to identify and prioritise research with the Great Barrier Reef Marine Park in terms of bioremediation;
3. to identify institutes capable of that research;
4. to establish links between government, industry, research and the community for such research;
5. to derive a statement on the application of bioremediation to the Great Barrier Reef in advance of a spill so that we have a policy statement ready when a spill 'occurs:

I trust that this workshop will be a usefu 1 one to all participants and thank you for participating.

Bioremediation - The Biological, Physical, and Chemical Bases

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The addition of microorganisms or the enhancement of the development of indigenous microorganisms with nutrient addition as methods for pollution abatement (bioremediation) have received a great deal of attention recently. Both techniques have been applied successfully in various laboratory and in situ remediation projects involving clean-up of water and soil in the United States for approximately the last 10 years (1). In some cases nearly complete removal and/or detoxication of pollutants have been reported (1). The United States Environmental Protection Agency has approved bioremediation for implementation at 24 National Priorities List sites (2).

Although bioremediation has become a viable remedial action technology in the U.S., the addition of broth or dried microbial preparations to natural environments remains a controversial subject. Moreover, the use of genetically engineered microorganisms for site clean-up is prohibited in the United Sates (3). The main cause for concern with genetically modified organisms is with the unknown fate of the organisms remaining after degradation of the chemical is complete and the potential for those organisms to somehow adversely affect public health. There is less concern with natural strains and indeed many companies currently exist which market a variety of organisms and assist in their implementation for site clean-up (4).

Even though bioremediation has developed rapidly as a viable technology there still exists a paucity of knowledge concerning its applicability and limitations. It is still not possible to predict the outcome of inoculation or nutrient amendment with only knowledge of substrate specificity and growth kinetics of the responsible organisms. Many other factors indigenous to the microbiological environment affect the efficiency and kinetics of treatment. For example, at a contaminated soil site degradation may he affected by the binding properties of the pollutants in the soil, the degree of mixing of the microbial cells and chemical(s) with the soil, oxygen availability (depth of treatment), the soil moisture content, presence of predatory organisms, temperature, availability of nutrients, and many other factors.

This paper provides a discussion of the physical, chemical, and biological bases affecting the efficacy of bioremediation for alleviation of contaminated sites. The significance of each is discussed and examples from the literature are provided.

1. Binding properties of the pollutants

It is well known that soils and sediments have the property of attenuating pollutants (5). Pollutants may remain suspended in the soil liquid and/or be adsorbed onto soil particles. There is evidence in the literature that adsorbtion affects the availability of pollutants to inoculate bacteria (6,7).

Toluene has been found to reversibly bind to soil in loosely and tightly bound forms. It is apparently degraded in the soil pore liquid after desorption from the soil particles. The loosely bound form is more readily available for biodegradation than the tightly bound form (6). The herbicide 2,4-D has also been found to be unavailable for biodegradation after sorption onto soil particles (7).

2. Degree of mixing of inoculated cells

Microorganisms are only able to mineralise a pollutant if physical contact is made with that pollutant. In aquatic environments chemical movement (and therefore mixing) is generally much faster than in soil. It is therefore likely that, if pollutants are biodegradable, inoculation or nutrient amendment of aqueous systems would lead to more complete exposure of the microorganisms to both pollutants and nutrients than in soil. All other factors remaining the same, one would expect faster and more complete biodegradation in aquatic environments than in soil.

Edgehill (8) has developed a computer model describing growth of inoculated bacteria on pentachlorophenol (PCP) in soil. A"micro-analysis" model based on removal kinetics corresponding to growth on PCP dissolved in the soil pore liquid with no adsorption correlated with soil inoculation data until 60-65% of the extractable PCP had disappeared. At later times, the model predicted more rapid and complete disappearance of the PCP than was observed experimentally. Residual PCP remaining in experimental soils may have been the result of incomplete mixing of the bacteria with PCP in the soil.

3. Oxygen availability

The availability of oxygen determines whether aerobic or anaerobic degradation of pollutants occurs. In the absence of oxygen (anaerobic conditions) biodegradation generally occurs at a much slower rate than when oxygen is present (9). Therefore, where possible, aerobic conditions should be promoted by limiting treatment to shallow soil or providing oxygen to contaminated anaerobic zones.

Crude oil, which contains 0.06 - 0.4% by weight oxygen, and its distilled products, require more oxygen for biodegradation than other more oxygenated pollutants. The theoretical oxygen demand of crude oil has been.reported to be 0.3 mg oxygen/mg oil (9). In spite of the higher oxygen demand, it is unlikely that oxygen limits biodegradation of oil confined to the ocean surface.

4. Presence of nutrients

Nitrogen and phosphorus are essential nutrients for the growth of bacteria (10). *Eschericha coli*, the model bacterial cell; contains 1.1, 3.2, 15, and 50% of sulfur, phosphorus, nitrogen, and carbon respectively (11). For biodegradation to occur nutrients will be assimilated in N/C, P/C, S/C mass ratios corresponding to those in biomass. Therefore nutrients must he added, if not already present, to ensure growth of the microorganisms on the pollutants.

Several commercial products are now marketed which contain microorganisms capable of degrading a wide variety of substances in addition to chemical nutrients (4). For' enhanced oil spill biodegradation oleophilic nutrients have been developed which

provide nutrients at the oil-water interface for biodegradation. Laboratory studies have indicated that, even with replacement of or unconfined seawater adjacent to the oil, nutrients remain affixed to the oil, do not trigger algal blooms, and are available to the microorganisms (12.13)

The United States Environmental Protection Agency has conducted field tests of Inipol EAP-22, an oleophilic liquid fertiliser developed after the 1978 Amoco Cadiz oil spill off France, and Customblen, a granular slow-release product for enhancement of oil spill biodegradation (14). Toxicity and eutrofication studies resulting from application of the fertiliser have also been conducted (14).

5. Microbial metabolism and growth rates

The complete conversion of an organic pollutant to inorganic products (C02, HCI, and water), mineralisation, is normally associated with growth of one or more organisms on the substance being mineralised. On the other hand, biotransformation and cometabolism are microbial processes in which the pollutant is transformed but is not itself utilised for growth (15).

Microbial degradation of crude oil has been studied extensively (16, 17). The crude oil fractions are amenable to biodegradation in the following order: aliphatics, aromatics, heterocyclics and asphaltenes. Some disagreement exists on whether aromatics or alkanes are more easily degraded (9).

Several organisms have been isolated which biodegrade linear alkanes containing as many as 44 carbon atoms (18). Branched and cyclic alkanes are more resistent to attack. Biodegradation of alkanes proceeds through intermediate carboxylic acids followed by their metabolism by beta oxidation.

Single, double, and three ring aromatic compounds are degraded by a variety of bacteria and fungi. Polycyclic compounds containing more than three rings are more resistent to biodegradation which may be related to their very low solubility in water (9). Very slow biodegradation of asphaltic compounds has been shown to occur in laboratory studies. However, in one experiment reduction of asphaltenes was attributed to adsorption onto biomass (9).

The growth rate or rate of biotransformation following inoculation or nutrient amendment strongly affects the kinetics of disappearance. Because microbial numbers do not increase with biotransformation, chemical disappearance rates are slow. Even with growth on the pollutant the disappearance rate will in general be slower than that corresponding to the maximum reported growth rate in free solution. Adsorption, limited contact of the pollutant with microbial cells, diffusion limitations, and the presence of predatory organisms all contribute to retarding the rate of disappearance in the natural environment.

The rate of pentachlorophenol (PCP) removal in soil by *Arthrobacter* strain ATCC 33790 was found to be less than in liquid culture at the same soil water concentration (mg PCP added to soil/l soil water). The lower rate in soil was attributed to limited contact of the cells with dissolved PCP in the soil water (8).

Biodegradation of oil has also been found to be slow in soil., Beach sediment containing from 0 to 50 g/kg oil is reported to have a biodegradation rate of 5-10 g/yr hydrocarbon. Based on these findings, heavily contaminated beaches may require 10 years for biodegradation if all of the oil is accessible for biodegradation (14). Lehtomaki and Niemala (19) found that introduction of hydrocarbon utilising bacteria, into soil had little or no influence on the residual oil concentration.

6. Solubility/availability of pollutant

Growth on immiscible or insoluble substances occurs at the water-organic interface or on substance dissolved in water (20,21,22). For substances of very low. water solubility, the availability of the pollutant for biodegradation is dependent upon inter-facial surface area. Many organisms produce emulsification agents or biosurfactants which disperse the substance and increase contact area availability of the pollutant for biodegradation (8). Commercial dispersants are also available to provide more surface area for microbial growth however these may also be toxic or increase the toxicity of the oil to marine life (23).

Efforts to artificially mobilise oil adhered to rocks have been met with limited success. As a result of the March 1989 Alaska oil spill cobble beaches became highly polluted with oil. Exxon proposed using Corexit 9580 M2, a kerosene-based solvent to solubilise the oil at rock surfaces. Although the solvent showed some effectiveness in removing the oil, recovery of the solvent-oil mixture was difficult. For this reason and the fact that there is incomplete knowledge of the toxicological properties of Corexit 9580 M2, use of the dispersant was discontinued.

Foght and Westlake (23) found that addition of Corexit 9527 temporarily retarded biodegradation of alkanes and had a variable effect on the utilisation of aromatics in media containing Prudoe Bay oil supplemented with nitrogen and phosphorus. It was not clear whether the delayed utilisation of alkanes was caused by toxicity to the bacteria or preferential utilisation of the dispersant (23)

7 Temperature

Microbial growth rate is a strong function of temperature and, if the Arrhenius equation is obeyed, should increase by a factor of approximately two for a rise of 10°C (23). The variation with temperature of degradation rate in the natural environment depends upon'the relative quantities of psychrophilic, mesophilic and thermophilic organisms in the environment. For example, the optimum temperature for hydrocarbon degradation is 20 - 35°C, although decomposition occurs over a wide temperature range varying from less than 0°C to 70°C (4,9).

The ability of the environment to degrade petroleum constitutes at low temperature apparently depends upon ambient conditions and the exposure history of the sample. Cooney et al. (24) found that aerobic biodegradation of four marker hydrocarbons was approximately the same at 0°C and 27°C in slurry samples taken from a portion of a lake with a past history of oil pollution. By contrast, low temperature was found to limit biodegradation in samples taken from nonpolluted portion of the same lake indicating that different microbial populations existed at the two locations (24). Atlas and Bartha found that winter samples of seawater contained high numbers of psychrophilic hydrocarbon degraders (25).

The temperature influence on degradation by quasi-pure cultures in natural environments is more pronounced. The half-life of pentachlorophenol (PCP) in soil at 300C inoculated with 10^6/g *Arthrobacter* strain ATCC 33790 was approximately 12 hours. On the other hand, soil inoculated with 10^5-10^6/g at an average ambient temperature of 12°C showed 50% degradation in approximately one week (8).

8. Presence of predatory organisms

Parasitism in natural environments is abundant (27). Bacterial numbers in soil and sewage are regulated by protozoa, vibrios and viruses (27). Studies have been reported in the literature on the effect of predatory organisms on the development inoculated bacteria in aquatic environments (28). Even though the possibility of inoculum die back with predation exists in some environments, total elimination almost never occurs (29). For soil environments, where mobility of both the predator and the prey are low, enough time may be available for the inoculum to accomplish significant biodegradation before loss of significant numbers. If the contaminated environment is infested with predators, repeated inoculations may be necessary.

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Bioremediation of Industrial Wastes

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Introduction

Bioremediation is a new- technique which is emerging as the preferred means of cleaning-up sites where organic compounds are the major contaminants. It involves using the existing microbial flora of the site (or sometimes introduced organisms) to bring about the conversion of these usually complex organic materials into simpler, harmless products such as carbon, dioxide and water. The microorganisms obtain the energy and the cell carbon they need to grow from the oxidation of the organic wastes. This process is called biodegradation.

Microorganisms have been known for many years to be involved in the breakdown of petroleum products, pesticides, and other complex organics in the biosphere. These microbes can metabolise organic pollutants in the soil, in natural waters or in engineered bioreactors.

Bioremediation is therefore a versatile approach to removal of hazardous pollutants from the environment, and has some significant advantages over other technologies:

- contaminants are destroyed, producing only C_2 and water as products
- the process is done on-site, eliminating the need for transport of hazardous materials or special waste holding or treatment facilities
- the process can easily be combined with other technologies for complex sites

While bioremediation has yet to be applied for marine oil spills in Australia, CRA has developed bioremediation technology which is finding application in treatment of a range of contaminated industrial sites, including gasworks and pesticide manufacturing plants. CRA has completed several bioremediation projects in Australia, and some of these case studies are presented below.

CASE STUDY 1 - A feasibility study at a gasworks in Sydney

The site is contaminated with coal tar and spent oxides, as well as some petroleum hydrocarbons. Coal tar is the major contaminant.

Soil from one of the more heavily contaminated areas was used. It contained 4000mgkg^{-1} (dry basis) total polyaromatic hydrocarbons (PAHs), and 2.0% total cyclohexane extractables, including petroleum-derived alkanes from C_{12} - C_{30} at 1000mgkg^{-1} dry soil.

Field Trial

25 tonnes of soil in 5 heaps received various treatments including inoculation with selected PAH-degrading cultures, nutrient addition (including N,P), water addition, and mixing or aeration (by forced air injection).

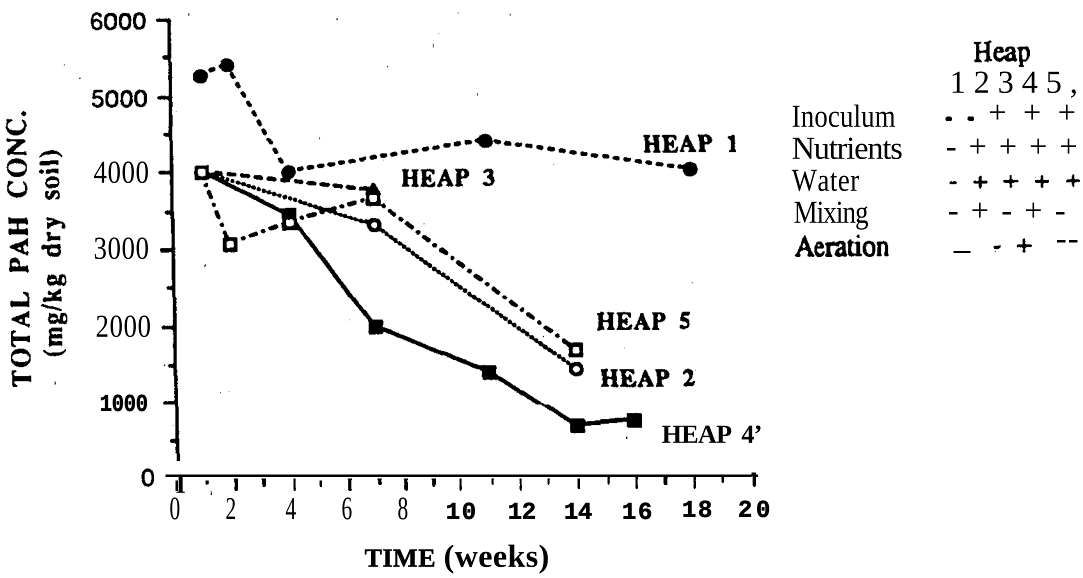

Figure 1. Biodegradation of PAHs in 5 tonne soil heaps

The most effective treatment resulted in 82% degradation of the total PAH fraction in 16 weeks.

Laboratory studies with this partially treated soil have also shown that an appropriate second phase treatment can increase the degradation rate, and reduce the residual PAH concentration from 750 to 300mgkg^{-1} dry soil in 4 weeks.

4-, 5- and 6-ring PAHs

The treatments applied resulted in degradation of all 16 PAHs studied. 2-ring and 3-ring PAHs were nearly completely degraded (>98%), but larger ring structures are more recalcitrant. Nevertheless significant removal of 4-, 5- and 6-ring PAHs was seen.

Table 1. Biodegradation of 4-, 5- and 6-ring EPA priority PAHs in soil.

	PAH Concentration ($mgkg^{-1}$)		
	4- ring	5-ring	6-ring
Initial level	1640	840	200
After heap treatment	265	310	95
After 2nd-phase treatment	83	150	53

CASE STUDY 2 - Commercial application to gasworks in Sydney

A gas holder contained 350 OOOL of oily waste including approximately 10 tonnes hydrocarbons as a heavy sludge.

This material contained 27% chloroform-extractable organics ("oil and grease") including n- alkanes from C_{12} - C_{22} and PAHs. A 30x25m treatment bed was constructed on the site consisting of a bitumen base and clay bund wall, coarse sand drainage layer and 0.5m depth of soil (see Figure 2a-d).

After transferring the sludge from the holder to the soil bed, inoculum and nutrient (nitrogen, phosphate) addition was begun, using cultures selected for sludge hydrocarbon degradation. Cultures were produced in large volume ($1m^3$) batches on site using a feedstock such as diesel supplemented with sludge. Tillage of the active surface layer (15-20cm) provided mixing and aeration. The sludge oil and grease was bound entirely to this surface layer. Water was added to the bed as required.

Initial total oil and grease of the active layer was 7.2%. After 130 days treatment this was reduced to 3.0% (Table 2). Analysis by an independent laboratory gave a final TOG in the active layer.(as freon extractables) of 1.43%. The average TOG content of the treatment bed was therefore reduced to 1.3% (or 0.4% above the measured background for this soil). The PAH content of the active layer was reduced from $1300mgkg^{-1}$ to $58.8mgkg^{-1}$.

Table 2. Total Oil and Grease (TOG) and 'Petroleum Hydrocarbons in Oyster Cove Treatment Bed

	Initial Conc.	Final Conc.
TOG (% $CHCl_3$ extractables)	7.2	1.4
TOG (% freon extractables)		0.5
Total Petroleum Hydrocarbons (%)	3.07	0.21
		(mgkg^{-1})
C_6 - C_9		nd[1]
C_{10} - C_{14}		40
C_{15} - C_{28}		1500
C_{29} - C_{36}		550
TOTAL PAHs	1300	58.8
Naphthalene	200	nd
Acenaphthylene	50	nd
Acenaphthene	210	0.65
Fluorene	80	nd
Phenanthrene	70	4.1
Anthracene	60	2.9
Fluoranthene	800	9.3
Pyrene	120	10.3
Benz(a)anthracene	90	4.6
Chrysene	110	4.3
Benz(b)fluoranthene	80	11.6
Benz(k)fluoranthene	60	nd
Benz(a)pyrene	70	5.2
Benzo(ghi)perylene	5	2.3
DiBenz(ah)anthracene	60	nd
Indenopyrene	5	3.4

CASE STUDY 3 - Pesticide?

Microorganisms capable of degrading pentachlorophenol (PCP) and 2,4-dichlorophenol (DCP) were isolated from soil samples from a Victorian pesticide manufacturing site. Since PCP and DCP are biocides, and are present at toxic levels in the soil, we have developed a continuous bioreactor system which can be used to treat either soil slurries. A biofilm reactor has also been used to treat simulated liquor from a soil washing plant (Figure 3).

[1] 1 nd = not detected

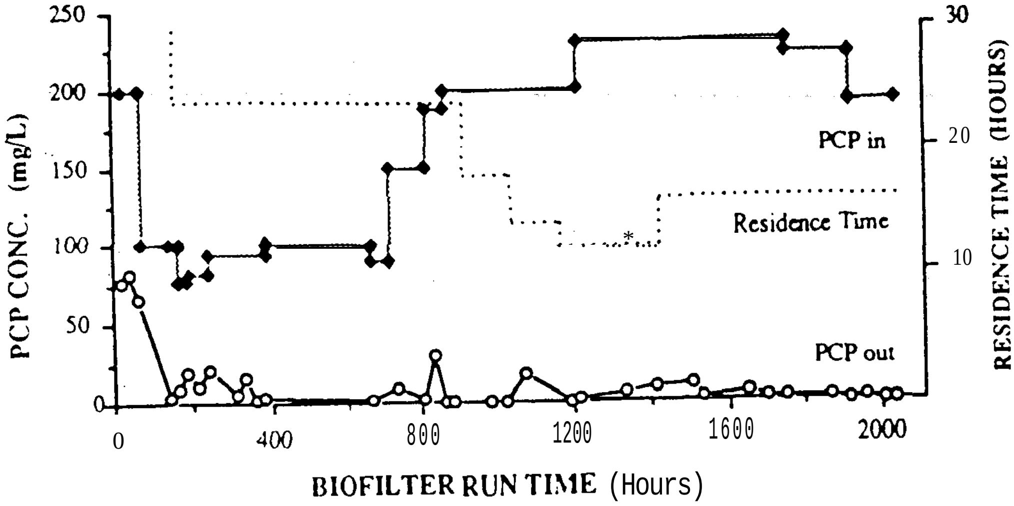

Figure 2. Biodegradation of pentachlorophenol (PCP) in a biofilter.

The graph shows the concentration of PCP in the feed and the discharge from the biofilter. A residual concentration in the discharge of $1 mgL^{-1}$ PCP has been achieved with a 16 hour residence time.

CASE STUDY 4 - Herbicides

An aquifer in Western Australia is contaminated with wastes from a herbicide manufacturing plant. A laboratory feasibility study is being undertaken to determine whether microorganisms capable of degrading 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are present in the aquifer or contaminated soil, and then to develop a bioprocess for decontaminating the aquifer.

CASE STUDY 5 - Diesel Spill

In September 1990 an estimated 500L of diesel fuel spilled from a ruptured above-ground storage tank at a food distribution company in Newcastle (NSW). Soil adjacent to the tank was contaminated, but most of the diesel flowed into a roadway gutter and down towards a Wetlands Reserve. Emergency measures were taken to repair the leak and contain the spilled diesel, and CRA initiated a rapid clean-up of the contaminated ground. The biotreatment was started within 36 hours of the spill, and consisted of excavating the contaminated soil to a contained area, and the application of nutrients and a culture of hydrocarbon degrading microorganisms. The selected culture was exceptionally efficient at degrading hydrocarbons of the types occurring in diesel fuel. Mixing and aeration was achieved using a rotary hoe.

Diesel hydrocarbon concentrations were reduced from 10000mg/kg to less than 1000mg/kg in 16 weeks. The soil showed no detectable odour or visual evidence of contamination after treatment, and was rapidly colonised by grasses.

CASE STUDY 6 - Oil Spill

A major spill of a high molecular weight lubricating oil additive occurred in early July 1990 north of Bulahdelah (NSW) on the Pacific Highway when a tanker overturned. About 15000L wasspilled, of which 5OOOL flowed into a steep gully. After an emergency clean-up, oil remained on the surfaces of the vegetation and rocks, and in the top few centimetres of the gully soil. The State Pollution Control Commission requested the assistance of CRA as the spill occurred in a sensitive State Forest, with potential long-term damage to the forest, and the likely slow natural rate of degradation.

Our assessment was that an accelerated biodegradation of the oil was possible, based on initial laboratory testwork. A selected culture was prepared, transported to the site and applied to the contaminated ground. Nutrients were also applied, and the soil was tilled, where accessible, with a small rotary hoe.

Results of the treatment have been encouraging, particularly in areas where tillage of the soil has been possible. A marked improvement in soil condition has been noted, and effects on the vegetation from the spill appear to have been minimised. The' treatment is continuing to be monitored.

DISCUSSION

These examples illustrate the development of bioremediation technology from the laboratory to commercial-scale activities. This technology will find application in many situations where organic pollutants are found in soil or water at industrial sites including

- gasworks and cokeworks
- oil refineries and fuel storage depots
- pesticide and herbicide manufacturing sites

We believe bioremediation processes can successfully be used to remove many organics, including some relatively recalcitrant contaminants such as the larger PAH structures found in coal tar.

Although these studies are all terrestrial applications of bioremediation technology,, they further our knowledge and experience in applying the technology at large scale. They also further assist in increasing public awareness and acceptance of the technology which would be a critical factor if the technology is ever to be used in sensitive areas such as the Great Barrier Reef Marine Park..

Bioremediation is rapidly emerging as a technique for clean-up of large scale industrial sites. As some older approaches to waste management disappear or become less attractive, so there is a need to replace them with cost-effective, efficient and environmentally sound alternatives. In the current climate, industry and the public are, impatient for new solutions to environmental problems. Provided that the scientific knowledge on which the development of bioremediation technology is founded is maintained and improved, it will evolve into a significant waste treatment option with the capability to deal with marine as well as terrestrial contamination events.

Research into Bioremediation of Oil and Related Compounds in Australia

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Introduction

Bioremediation has been described simply as "Biological oxidation of hazardous waste, using bacteria and other microorganisms" (O'Gallagher, 1990). Oil and petroleum are certainly environmentally toxic substances and the remediation of terrestrial sites contaminated with these and related products using microorganisms (with or without added nutrients) is a process that has been used in many overseas countries for over twenty years and has been shown to be economical, efficient and environmentally sound (Vellacott, 1990). In comparison, the application of microorganisms and/or nutrients to remediate oil or petroleum spills in the marine environment has for many years been viewed with extreme caution due to concerns about unknown long-term effects, possible toxicity to nearshore environments and general effectiveness. However, failure of other methods to combat the massive oil spill produced during the Exxon Valdez disaster in 1989 finally led to approval being granted (in the USA) to employ bioremediation techniques to clean-up oiled beaches in Alaska, and later in Galveston Bay, Texas, after another tanker accident (LeBlanc and Fitzgerald, 1990; Anon (a), 1990).

Bioremediation of oil and related compounds in Australia is being seen as a "new technology", having been carried out only within the last few years on terrestrial sites with much success. The research involved in the development of application techniques and other processes to enhance bioremediation in both terrestrial and aquatic situations in Australia, including the Great Barrier Reef, is the focus of this paper.

Research in Australia as background to potential use of bioremediation in marine environments

Oil is composed of many hydrocarbons, the presence and proportion of which vary depending on the origin of the oil and degree of refining. To enable selection of the most efficient response option to combat an oil spill in the marine environment it is essential to know the type of oil spilled. This is especially important when bioremediation is an option to be considered. Subsequent to the success of bioremediation on shoreline clean-up operations in Alaska and Texas, authorities in these states declared that this method is the preferred and in some cases the only option they will consider for future oil spills (Alan Sheehy, pers comm; Anon (b), 1990). These authorities have recognised that not only must the chemical composition of the spilled oil be determined, but also the nature and size of the resident microbial populations and nutrient levels at the polluted site (LeBlanc and Fitzgerald, 1990).

Although, to the author's knowledge, no experimental research involving bioremediation of oil on open water or shoreline environments has been carried out

within Australia, there have been numerous studies conducted within the last ten years in various locations (including the Great Barrier Reef) that have determined baseline levels of hydrocarbon-degrading marine microorganisms. Concurrent measurements of tptal hydrocarbon loads also,showed that microbial bioassays are useful and relatively inexpensive markers of hydrocarbon levels in the environment (Sutton et al., unpub. data; Hay, 1983; Larsen, 1986).

There is also a well-established collection of methodologies in laboratories throughout Australia for analysing environmental samples for hydrocarbons. Techniques commonly used include capillary GC, GLC, HPLC and GC/MS for detailed composition of aliphatic and aromatic components, and distinction between biogenic and petroleum hydrocarbons, and more recently Iatroscan TLC-FID for rapid measurement of the total hydrocarbon load (Volkman et al., in press, Jan; 1991; Tabak et al., 1990). Studies carried out by a number of researchers in Australia (Hay, 1983; Dunlop and Jeffries, 1985; Volkman et al., 1988, and 1991 in press) have emphasised the importance of determining the distribution and abundance of biogenic hydrocarbons in both coastal and offshore (i.e. mainly reefal) environments. Some authors have postulated that the presence of such compounds, notably in sediments, may account for the ready detection of both n-alkane and polycyclic aromatic hydrocarbon (PAH) degrading marine bacteria in even remote (i.e. pristine) environments (Hay, 1983; Larsen, unpub. data).

Another aspect of research conducted in Australia that is especially relevant to possible in situ bioremediation in marine environments is the isolation and maintenance of stocks of naturally-occurring hydrocarbon-degrading microbial cultures. As will be discussed shortly, the teams actively involved with bioremediation of contaminated land, oily sludges and fuel storage depots, etc., have been accumulating collections of highly active degradative microorganisms, as strains specifically isolated from each site are used in preference to allochtonous (non-indigenous) species. In addition to these stocks is the Australian Collection of Marine Microorganisms (ACMM) maintained at the Sir George Fisher Centre for Tropical Marine Studies at James Cook University in Townsville. Held within this Collection are a large number of marine hydrocarbon-degrading bacteria, including both pure cultures and communities that can degrade both aliphatic and aromatic hydrocarbons. These cultures are the products of various research projects carried out within Queensland, and are of special significance to potential bioremediation within the Great Barrier Reef region a's they represent microflora indigenous to local tropical coastal and reefal environments.

It is clear from this discussion that there are a number of research facilities within Australia with the background and capabilities required to not only rapidly assess the site/spill information necessary to enable selection of the most suitable bioremediation technology, as is being recommended by the United States Envionmental Protection Agency (LeBlanc and Fitzgerald, 1990) but to also enable rapid implementation of the method of choice.

Research in Australia on enhancement and application of bioremediation of oily wastes

The research programs mentioned above, however, were not conducted with the sole objective of developing bioremediation technologies. There is, although, an oft-expressed view that: "Whilst many laboratory scale technological advances (in waste management) are continually being made, few of these appear to move on to commercialisation". This observation is quoted from a recent review concerning Australian capabilities and technological developments in liquid waste management (O'Gallagher, 1990) and may help to explain the lack of available information and consequently the low level of public awareness about the research that has been conducted within Australia on oil pollution bioremediation. The review itself was instrumental in identifying the few major centres in Australia that are doing pioneering research into this rapidly developing field.

These research centres (and a few others) are listed in Table 1. The projects within the first two cent-t-es are being conducted in collaboration with other (Australian and International) companies and institutions. CRA's Advanced Technical Development section and Genesearch are two lab-based Australian companies independently carrying out research and development, and there are a number of other companies involved more with the application and monitoring of on-site oil farming technology than with research. These are exemplified by Scott and Furphy in Sydney and a number of industries in Western Australian areas including Kwinana and the Pilbara region. The Western Australian Environmental Protection Authority, along with environmental consultants in WA have been a force behind the implementation in that state of oil farming oily wastes i.e. emulsified oily liquids that are non-recyclable (Vellacott, 1990). --

The research programs listed in this Table show that most research in Australia is concerned with bioremediation of collected or waste oil and other hydrocarbon-containing substances using processes involving bioreactors or digestors, biofilters or land applications (oil farming). Only one project is being undertaken with the aim of using bioremediation to treat spilled oil in a marine environment (Genesearch's BIOMARINE-PLUS project). In situ applications to spilled oil on the open water and shorelines have not yet been trialed in Australia, as Australian authorities are skeptical of the effectiveness of these newly demonstrated techniques, just as officials in the US were, prior to the Exxon Valdez spill clean-up operations last year.

Profiles of these major research activities, including in some cases how they were initiated by the researchers involved are given below.

Dr Alan Sheehy joined the University of Canberra in 1979 before going to the Bureau of Mineral Resources (BMR) where he worked with Baas Becking who headed the Geobiological Laboratory there. This laboratory was jointly run with the CSIRO. When the lab closed in 1986 Dr Sheehy moved with other members of the Petroleum Section of the facility back, to the University of Canberra, where'in 1987 the Microbiology Research Unit (MRU) within the Faculty of Applied Science at the University was established. As with the BMR this Unit is a joint facility with the CSIRO. Initially Dr Sheehy's work concentrated on the enhancement of oil recovery using microorganisms, as well as corrosion studies, focussing on selective microbial degradation of coals and petroleum. Although the MRU is still conducting research into, and implementing field programs for improved oil recovery using microorganisms, it has become heavily involved in biorestoration of contaminated sites and bioremediation of toxic and hazardous wastes.

The MRU's fundamental research concerns in situ bioremediation of sites contaminated with a variety of toxic compounds. A total of nine contaminated sites within five states are currently being decontaminated; seven via excavation and construction of soil bed /clay layer upon which appropriate bacterial cultures and /or nutrients are applied, and two via in situ remediation, i.e. application without excavation. Contaminants treated on-site include coal processing products, sludges, petroleum and petroleum derivatives and nitroaromatics. The bioremediation processes developed by the MRU use only microorganisms that have been cultured from the site to be remediated and are therefore adapted and efficient at degrading the pollutant material: As will become evident later, it appears that Australian bioremediation researchers have used and particularly advocate this protocol of using only indigenous microorganisms in their applications. Periodic addition of nutrients augments the metabolism of the added, inoculum (ie., large scale cultures grown onsite in bioreactors) subsequently speeding up the bioremediation process.

Although commercial application of bioremediation to contaminated sites is being undertaken by the MRU, mostly in collaboration with other agencies and companies (eg., BHP and CSIRO), it is the enhancement of biodegradation within bioreactors that constitutes the practical research concentrated on at the MRU. Dr Sheehy maintains that polluted material being degraded in bioreactors needs organisms that persist through the changes that naturally occur in a closed metabolic system. His team is thus investigating these changes in natural populations of biodegradative microorganisms, ie., studying the natural evolution of the community. As bioremediation is often a slow process, this research is working to pinpoint what these changes are and how they occur to enable the most effective microorganisms to persist as long as is required for decontamination of the waste substrate. In conjunction with these studies is a project being undertaken by BHP to evaluate the changes occurring within the hydrocarbons, during natural degradation. This work involves detailed chemical analyses and is being conducted by Dr Evan Evans at the BHP Research Laboratories in Melbourne. Their next major project will be concerned with identifying metabolites of PAH oxygenation under bioremediation conditions - the results of which are of obvious relevance and importance to the microbial studies.

The MRU has also been involved with the bioremediation project carried out by Exxon on the shorelines of Prince William Sound, Alaska, after the Exxon Valdez oil spill. This project involves the development of a model to determine the response of organisms to the impact of an oil spill under varying environmental conditions. Of major consideration is whether contaminated shorelines consist of high or low energy beaches, as obviously high aeration increases biodegradation. Dr Sheehy has been involved with the production of wave simulators to enable such studies to be carried out. He has stressed that the most problematic situation occurring with marine oil spills is contamination of fine sediments occurring in coastal and estuarine areas, e.g. mangrove environments. Adherence of oil to silts and clays is very strong, thus limiting response plans in such areas to protection or prevention, a point worthy of some note in regard to future research priorities in Australia.

Professor John Waid of the Department of Microbiology at the La Trobe University in Melbourne, has been heading a team that have developed a commercially viable system for the bioremediation of organochlorines using white rot fungi. With the assistance of Saftec Pty Ltd, an Australian firm, the process is currently being improved further in regard to application of inoculum to contaminated sites, and, interestingly to develop bioreactor technology to degrade bulk organochlorine wastes.

Professor Waid is also acting as a microbiological consultant to a project being carried out in Melbourne by Brightwater Technology Pty Ltd, operated by RMS Consultants, a civil engineering firm. This project is using an enhanced bioremediation process to rejuvenate oil-contaminated silty sludge. The sludge is being produced from a patented soil-washing process used to clean 150 000 m3 of oil-polluted sandy soil at Bayside, Melbourne. The properties of very fine silt as mentioned above, mean that the sludge retains petroleum hydrocarbons in much higher concentrations than does the sandy soil. The company belives that the scale of this project will highlight the potential of their technology, as it has been estimated that 5000 m3 of sludge will be produced, i.e. 5 million litres. The process is carried out on batches of 80 000 litres in bioreactors fitted with immersed heaters and aerators located on the bottom. Hydrocarbons with low boiling points and odiferous compounds released via volatilisation during the initial stages become oxidised by an emission combustion unit (Figure 1). Bacteria in the sludge oxidise the remaining heavier hydrocarbons until the levels fall below environmental acceptance criteria. About 15 m3 of sludge is used as inoculum for the next batch. According to Noel Murphy of RMS Consultants the total process can be as short as several days, depending on the initial level of contamination, and the final product is used to form topsoil by mixing with clean sand from the original site. Here again, no exogenous microorgasnisms are used in the process.

RMS Consultants are a civil engineering firm that have a background in heavy mining and the offshore oil industry. The ideas behind this bioremediation enhancement process arose after discussions with members of other geotechnical and environmental firms, and treatment of the first batch of sludge in the Bayside project began during February this year. It would be of great interest to compare costs and time efficiencies of this combinaton of physical and biological processes to other bioremediation techniques in use within Australia such as those implemented by the CRA's ATD and the MRU at the University of Canberra.

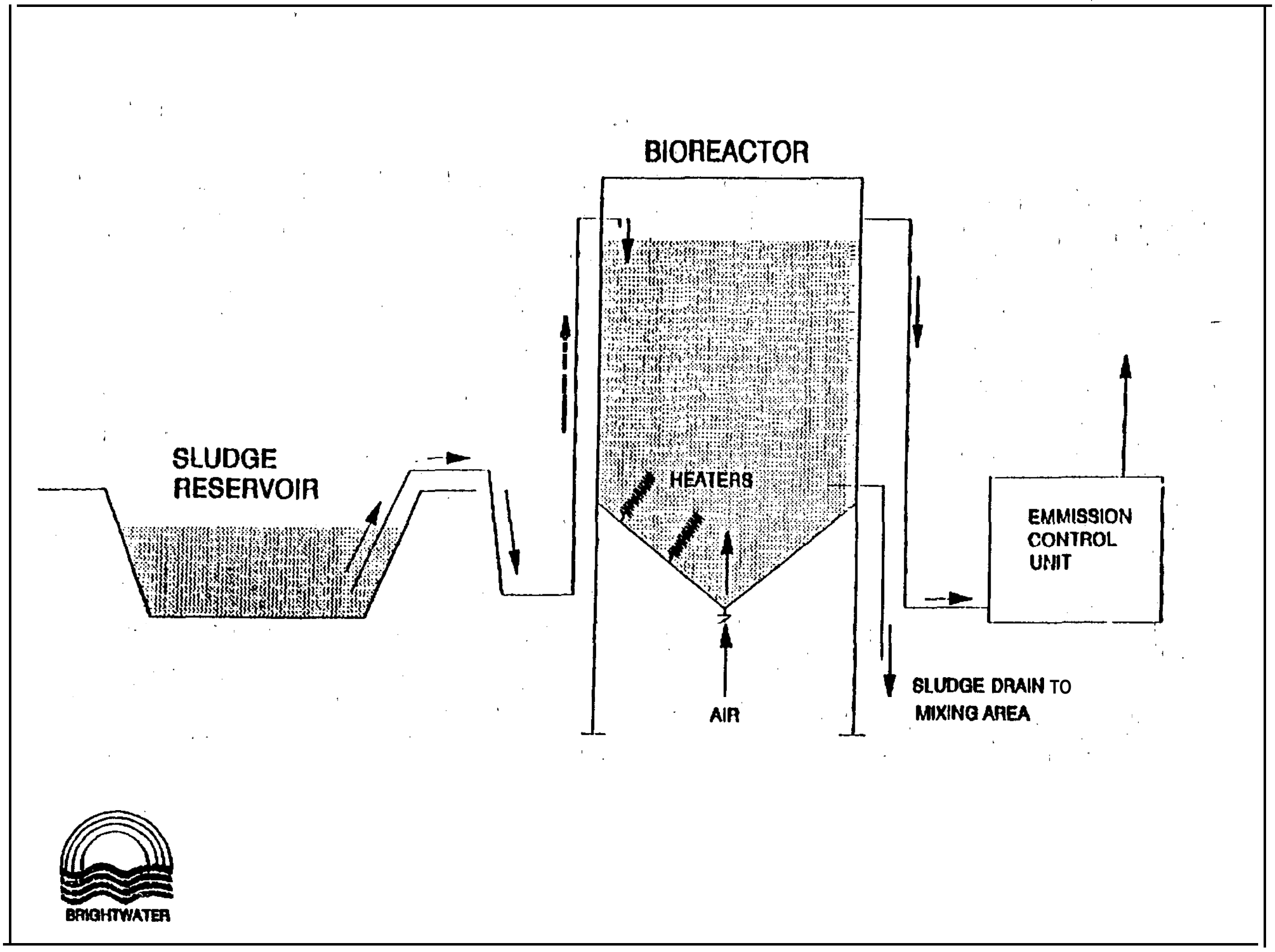

Figure 1. Sludge bioremediation layout developed by Brightwater Technology Pty Ltd

Dr Bruce Kelley is the research Manager of the Advanced Technical Development (ATD) team of the CRA company and is involved with bioremediation research into chlorinated phenols, phenoxyherbicides and creosote-contaminated materials (in association with the timber industry) as well as treatment of oily wastes, coal tars and PAHs. The largest commercial bioremediation project successfully completed by the ATD used a combination of nutrients and highly active microbial cultures to remediate 400 000 litres of a water/oily sludge mixture. During this process, no heating, washing nor extraction processes were involved.

The various bioremediation projects of contaminated industrial sites carried out by CRA have resulted in the isolation of a large stock of microorganisms capable of degrading a range of organic pollutants. Dr Kelley's team have also been conducting research into understanding what physico-chemical requirements are necessary for maximal mineralisation rates of organic pollutants.

Scott and Furphy are a Consultant Engineering firm who are also currently involved in a bioremediation project. According to Ian Law of the Sydney branch of this firm; their on-site remediation of a disused oil refinery in Sydney is less costly than the soil-washing process discussed above. Their system involves firstly extraction of volatile hydrocarbons from the contaminated soil using activated carbon, before land farming'

on-site. They are not adding any nutrients to the soil but are tilling and keeping the bed damp. As they admit, this process is very slow, and they may inoculate before the onset of the cooler winter months. The firm have their own microbiologists who have conducted bacterial counts, etc. in the contaminated soil for a number of years prior to the initiation of the remediation process.

Dr John Reichelt is the director of Genesearch, the other major company involved with research and development of bioremediation processes in this country. Genesearch was formed in 1981 by scientists formerly working at the Roche Research Institute of Marine Pharmacology, Sydney (RRIMP). One of Dr Reichelt's early major interests was designing and constructing fermentors for production of high biomass microbial cultures. He was also very interested in microbial degradation of organic pollutants, having been a research associate of the Sir George Fisher Centre since various projects determining the distribution and abundance of hydrocarbon-degrading marine bacteria and various hydrocarbons in tropical marine environments began at the Centre in 1984.

Genesearch's BIOTOX process was designed for elimination of PCBs and is, like the Brightwater Technology Pty Ltd remediation system, a combination of both physical and biological processes that together completely mineralise the pollutant substrate. BIOTOX has already been patented for use, both in Australia and overseas for use on bulk PCB waste. The system uses bacterial degradation after pre-treatment of the PCB-containing waste with UV irradiation. The irradiation breaks down chlorine atoms from the aromatic components thus providing a hydrocarbon substrate much more susceptible to bacterial attack. A consortium of bacteria isolated from soil are used, and complete the degradation process.

The first process developed by Genesearch as a full scale system was their ECOBAC process for digestion of grease-trap waste. The company designed, built and trained personnel to run a plant in Brisbane that digests 80 000 litres of waste per day, and plans are underway to build a second plant in Sydney capable of handling 200 000 litres per day.

The ECOBAC process (along with other marketed bioremediaton products for the same substrate) is an enormous improvement on the previously used system of burying the waste, where, with a pH of 4.5, natural biodegradation was either inhibited or extremely slow. In the ECOBAC system, a mixed culture of bacteria grown on grease are concentrated to a stable powder formulation which is used to dose a series of digestors. The bacteria are allowed to flourish, and as stationary phase is reached, the digestor is dosed again, and this procedure repeated. The final product is used as a soil improver - an added benefit.

It was out of the ECOBAC project that Genesearch's current research arose: the development of a product known as BIOMARINE, and just recently BIOMARINE PLUS. The former is a bacterial formulation used to eliminate visible oil from bilges. As it was found that the remaining water contained a high load of soluble organics (eg. phenols), a second formulation was developed to remove them. This latter process is still at the pilot plant stage, but the final product (BIOMARINE PLUS) represents the first bacterial formulation that is applicable for in situ bioremediation of spilled oil in marine environments.

It is hardly surprising that, Australian'researchers have not'been hurriedly pursuing experiments to apply bioremediation to controlled oil "spills" in field situations or. simulations, given the overwhelming feeling internationally against its use over the last decade, due to uncertainty of usefulness shown in early overseas experiments, and, 'possible toxicity of additives., The idea of 'seeding' oil spills with microbial inocula has been'around since the mid 1970s and studies concerning addition of nutrient fertiliseis were published as early as 1973 (Bartha, 1986). Whilst these techniques'were viewed as having potentially toxic or disruptive side effects, it was the escalation of research into genetically-manipulated*microorganisms during the 1980s that led to worldwide concern over release of such unnatural organisms into delicately balanced marine ecosystems, and exacerbated the anti-bioremediation sentiment. However, scientific and industrial opinion (in the USA at least) is poised to tip the scales in favour of bioremediation, due to the success of the shoreline bioremediation experiments in Prince William Sound during mid-1990 (which involved no additions of allochthonous microflora) and to a lesser extent the first open water bioremediation experiment on a spill produced after an explosion onboard the Mega Borg crude oil carrier in the Gulf of Mexico (also during mid 1990). The inoculum added to the latter spill was a mixed culture of non-indigenous bacteria produced by Alpha Environmental Inc., from Austin, Texas. Results from the former program showed that treated areas were cleaned to 30 cm below the beach surface, with no toxicity to native shrimp nor production of algal blooms reported in nearshore waters, whilst results from the latter experiment were reported by some to be inconclusive, as wind and wave action did not allow accui-ate results to be obtained (LeBlanc and Fitzgerald, 1990; Anon (a), 1990).

Although application of bioremediation to in situ marine oil spills is still in its infancy, its potential as an effective and economically (and most likely environmentally) sound clean-up option is undeniable. Given the.earlier examples of both the quality research already taking place and obvious potential of research organisations within Australia, it is clearly becoming very timely, and indeed essential, for appropriate research to be initiated into developing bioremediation technologies applicable to oil spills in tropical Australian marine environments.

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Acknowledgments

The generous help provided by Alan Sheehy, Bruce Kelley, John Reichelt, Noel Murphy and Steve Vellacott during the preparation of this paper is gratefully acknowledged Thanks also PO to Michael Warne, Analiese Caston and John Volkman for their valuable assistance.

Bioremediation Applications in the Great Barrier Reef Marine Park Summary of Presentations

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Summary

From the various talks that were presented at the workshop, it is clear that a number of unresolved issues remain on bioremediation and its application in the reef environment. 'These issues in turn lead to a number of research objectives to address these issues. Possible research directions have both a strategic and tactical component to them and will require the Great Barrier Reef Marine Park Authority to be a major player in the management of any programs if they are to be focussed on reef-related problems.

Some possible short-term research issues which were suggested by the workshop are also identified.

With respect to the application of bioremediation to oil-spill clean-up, it appears that there are four possible areas to which microorganisms might be applied:

(1) an uncontained body of water,
(2) a contained body of water,
(3) coastline (beaches etc.)
(4) coral reefs

Categories (2) and (3) appear to have greater short-term potential.

TABLE 1

RESEARCH OBJECTIVES

1. To know if and when bioremediation is feasible

2. To know how to utilise bioremediation effectively

3. To have supplies of bioremediation agents either available or capable of being generated

4. To have ensured community is aware of and accepts bioremediation

TABLE 2

BIOREMEDIATION QUESTIONS

1. ROLE OF BIOREMEDIATION

- marine environment
- groundwater environment
- solid environment

2. EFFECTIVENESS OF BIOREMEDIATION

- organisms (genetics, metabolism)
- environmental conditions (nutrient addition)
- contacting, mixing
- time constant
- cost
- physical, chemical, biological factors

3. ENVIRONMENTAL CONSTRAINTS

- nutrients
- residual/non-biodegradable
- intermediates
- genetically modified organisms

4. SOCIAL CONSTRAINTS

- genetically modified organisms
- naturally occurring organisms
- regulatory bodies

TABLE 3

RESEARCH DIRECTIONS

1. ROLE OF BIOREMEDIATION

- Critical review - bioremediation in warm water
- Assessment of environmental constraints

2. BIOREMEDIATION AGENTS

- Screening, metabolic pathways
- Identification
- Optimise growth/performance
- Analytical issues

3. APPLICATION OF BIOREMEDIATION

- Controlled trials
- Develop a protocol
- Integrate models
- Synergistic treatments

4. SOCIAL ACCEPTABILITY

- Establish community response

TABLE 4

RESEARCH MANAGEMENT
(with respect to GBRMPA)

(1) GBRMPA to act as a broker for research relevant to the Great Barrier Reef Region

i.e. to put together a consortium, of researchers, obtain funding and manage program (e.g. AMIRA)

(2) Need for both STRATEGIC and TACTICAL research programs

e.g. RNA based probes for identifying specific organisms (STRATEGIC); optimising degradation of PCBs (TACTICAL).

(3) Need for a PROCEDURES document

(4) Establish links with other remediation/control actions.

TABLE 5

POSSIBLE SHORT TERM RESEARCH NEEDS

1. ASSESSMENT OF IMPORTED OR LOCALLY PRODUCED ORGANISMS

   - Effectiveness - ideal conditions
     - field conditions
   - Safety - immediate
     - long term
   - ♍□⬩◆⬩

2. NETWORKING OF LOCAL EXPERTISE

   - Sub-critical mass currently exists
   - Range of problems is wide; frequency is low

3. STATE OF ART DOCUMENT, IDENTIFYING IN MORE DETAIL RESEARCH NEEDS AND OBJECTIVES

Bioremediation of Oil 'Spills

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Introduction

Petroleum is a complex mixture consisting predominantly of hydrocarbons. The hydrocarbons can be divided into four groups based on molecular structure. The groups are aliphatics, linear forms either straight or branched; cycloaliphatics, sometimes with side chains; and cycloaromatics, a combination of aromatic and cycloaliphatic structures. Overall hydrocarbons range from gases such as methane. through liquid fractions such as petrol to solid bitumens such as those used to make roads. Hydrocarbons are of extreme economic importance because of their central role in energy production.

The presence of hydrocarbons in the environment is of major concern as even small amounts of hydrocarbons (mg/l) may act as toxins, mutagens and cascinogens. Once in the environment, hydrocarbons spread into inanimate and biological systems. Biological systems will often accumulate (bioaccumulate) rather than metabolise hydrocarbons. Accumulation is not limited to to primary ingestion of hydrocarbons but can occur through consumption of biota containing bioaccumulated hydrocarbons. This effect is known as biomagnification and can affect all members of the food web including humans. In general, the longer hydrocarbons persist in the environment the greater will be the risk to the ecosystem and the more difficult they are to eliminate from the area.

Significant sources of hydrocarbons in the environment are seepage from petroleum reservoirs, run-off from road surfaces, and accidents that occur during the manufacture and transport of oil. The vast majority of these hydrocarbons are destroyed without human intervention by natural microorganisms through a process called biodegradation. In this process hydrocarbons and related compounds are converted to harmless cellular components such as fatty acids. These natural processes continue whilst the amount of hydrocarbons entering the environment is below a critical level of carbon. Above the critical level, the microbial ecosystem which is responsible for degradation is destroyed. Whilst only a small fraction of the total hydrocarbons entering the environment occurs through transportation accidents, spillage of large volumes of petroleum or spillage into an ecologically sensitive areas such as the Great Barrier Reef Region can cause catastrophic results.

Bioremediation is the term applied to human augmentation of biodegradation. In this process, the growth of natural or cultured microorganisms is promoted in a contaminated site. In this way the ecological balance is restored in favour of the microrganisms and the containment destroyed. The processes used in bioremediation have been used for nearly a century to remove organic material from sewage. In the past twenty years it has been used to clean-up'hydrocarbon contaminated aquifers.

Bioremediation was used to clean the shoreline areas of Prince William Sound (Alaska) commencing in 1989. This was the first systematic, large scale use of bioremediation to offset the effects of an oil spill. The success of bioremediation surprised public officials and environmentalists. At this time bioremediation is the only technology permitted by the United States Environmental Protection Agency and Alaskan authorities for the restoration of Prince William Sound shorelines. The second large scale use of bioremediation occurred in the Gulf of Mexico (USA) in 1990. A commercial preparation of dried microorganisms was applied in an open sea trial of bioremediation. The results and interpretation of the results remains controversial. However, the results were conclusive enough for Texan authorities to make bioremediation the centre of oil spill contingency plans.

Abiotic and biotic influences on spilled oil

Petroleum spilled in the marine environment will undergo changes from abiotic and biotic influences. The initial size and distribution of the slick will be the result of **wind,** wave and tidal action. As the slick spreads, volatile hydrocarbons evaporate and low molecular weight aliphatic and aromatic hydrocarbons will solubilise. The natural microbial population begins to attack alkanes and low molecular weight aromatics in combination with abiotic oxidations. During this period, the appearance of the slick may alter if wind and wave action causes the formation of emulsions, particularly if oil in water emulsion (mousse) is formed.

The processes of solubilisation, volatilisation, photochemical oxidation, emulsification and microbial attack are known collectively as weathering. As weathering proceeds, oil becomes more viscous and more dense, leading to the formation of tarballs. Tarballs are distributed throughout the water column and sediments. Both tarballs and emulsified oil may sink and later rise to form further slicks. As weathering proceeds, the most readily metabolised hydrocarbons are removed and the oil becomes more resistent to microbial attack. The most resistant hydrocarbons will sink and reside in the sediment. In general, the lack of oxygen in the sediments will limit further microbial attack. Wave action may result in unweathered oil reaching the sediments. This may be the cause of subsequent beach contamination.

During the initial response to an oil spill the use of dispersants frequently is advocated. There are polar views on the use of dispersants as an oil spill response. Emulsions created by dispersants generally drop oil to the bottom of the water column and into sediments where natural biodegradation would be slowed by the absence of oxygen. There is also considerable speculation that dispersant-petroleum combinations are more toxic to the naturally occurring microorganisms which would normally biodegrade the contaminant hydrocarbons.

Bioremediation and the Great Barrier Reef

To understand the potential for applying bioremediation within the Great Barrier Reef Marine Park, it is necessary to appreciate the diverse composition of petroleum, the range of microorganisms in the ecosystem impacted, the physical and chemical status of the area, and the sequence of events which occur after oil is spilt into the marine environment. Without this understanding, degradation of oil in the Reef environment can be considered the destruction of crude oil of unknown composition by an unknown

consortia of organisms in an erratically changing environment. This is not consistent, with good management practices for the Reef.

In any consideration of the applicability of bioremediation to the Great Barrier Reef it is important to assess the natural biodegradative potential of the region. To date, naturally occurring bacteria capable of degrading all hydrocarbons have persisted in the environment, either physical or chemical. factors have been responsible for the suppression of biodegradation rather that an absence of appropriate microbial strains. The logical approach would be to investigate which factors suppress the natural biodegradative potential in the Reef.

There would seem to be no justification for introducing microorganisms which are, not native to the Reef area. This is supported by the findings at Prince William Sound. Within six weeks of bioremediation stimulation the natural microbial population increased 100 fold, natural organisms in the treated area degraded oil at three times that of the untreated area, and shoreline areas were cleaned to 30 cm below the beach surface and 60 cm at high wave energy sites. Peak concentrations of nutrients, 0.6 mg/l ammonia and 1.6 mg/l nitrate were not toxic to marine organisms and no algal blooms resulted.

There is a clear role for bioremediation in the Great Barrier Reef Marine Park. After rapid physical removal, bioremediation is the method of choice for treatment of contaminated beaches, contained water bodies and as a disposal mechanism for oil recovered from skimmers. No immediate option exists for bioremediation of hydrocarbon fractions floating on the surface, in the water column, associated with the Reef structure, and in sediments though laboratory studies and field trials are promising.

The Exxon Valdez Oil Spill - Woodward-Clyde Consultants' Contributions to Bioremediation

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Approximately eleven million gallons of crude oil were spilled into Prince William Sound, Alaska, when the Exxon Valdez supertanker ran aground in March -1989. The ensuing massive clean-up operation utilised a wide variety of conventional techniques, including high and low pressure spraying, specialised skimming equipment, and hand scrubbing of oiled rocks. These methods can be very damaging to the local environment and are not capable of capturing and removing all oil trapped within beach sediments.

Within days of the oil spill there was an increase in the populations of microorganism capable of utilising carbon in the oil as a source of food, and consequently degrading the oil to carbon dioxide, fatty acids and water in the process. The United States Environmental Protection Agency (EPA) recognised the possible role of microorganisms to clean-up oil trapped in the beaches and considered that this type of bioremediation could enhance the clean-up effort. Following review by an expert committee recommendations were made to undertake field testing of bioremediation on affected beaches.

Microbial activity in surface water and groundwater can be inhibited by lack of oxygen, unfavourable redox and acidity conditions, poor nutrients balance, mass transfer dynamics (mixing) and extremely high concentrations of the pollutants to be degraded. The microbial ecology approach to bioremediation seeks to adjust the environmental factors, e.g. oxygen and nutrient availability, to maximise microbial productivity and pollutant upgrading the plant.

Microbial activity can be stimulated by addition of essential components in proper combinations which in turn will enable the microbes to multiply and feed on the organic pollutant which is consequently degraded.

Woodward-Clyde Consultants' approach to bioremediation is to utilise naturally occurring microorganisms and to adjust their environment to achieve optimum productivity. This 'microbial ecology' approach has been successfully utilised by Woodward-Clyde Consultants to remediate a number of sites contaminated with organic solvents including removal of dissolved aromatic hydrocarbons in treated ballast water at the Ballast Water Treatment Facility (BWT) at Port Valdez.

In this case microbial activity was enhanced by introducing additional oxygen by aeration, with a consequent reduction of concentrations of BTEX and polyaromatic hydrocarbons (PAHs) and a saving of many millions of dollars on upgrade.

Microbial activity on the oil polluted beaches in Prince William Sound was found to be inhibited by lack of nitrogen and phosphorus nutrients. Successful field testing in 1989 of oleophilic fertilisers, which adhere to oil covered surfaces thereby making nutrients available at the site of microbial activity, led to a request by EPA for "Bioremediation Assistance" announced on 12 February 1990. Thirty-nine proposals were submitted, of

which eleven, including that of Woodward-Clyde Consultants, were selected for further study.

The present situation is that approximately 190 kilometres of Alaskan shoreline remain moderately to heavily contaminated with considerable oil penetration of the beaches. The residual crude oil is now an insoluble tar, The Woodward-Clyde Consultants proposal was based on its assessment of the contamination situation and principals of bioremediation by microbial ecology. The proposal advocated the use of an emulsifier (Toxigon 2000), Medina Soil Activator, and MAX BAC slow release nutrients, all to be applied sequentially by spraying from a helicopter.

The proposed bioremediation approach, if approved, will require EPA Assessment of pilot programmes which will include toxicity/efficacy tests of the various commercial products to be applied, and resolution of the issue of which indigenous microorganisms should be supplemented by exogenous forms.

It appears evident at this stage that bioremediation offers the only effective treatment for oil contaminated sediments in Prince William Sound where their physical removal' is impractical.

PAPERS SUBMITTED

Biodegradation of Oil in the Open Ocean

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*In reply to a request from the Coast Guards National Response Team, the Environmental Protection Agency (EPA) has asked the American Society for Microbiology (ASM) to advise on the state **of** the science **of** biodegradation **of** oil in the open ocean, and specifically to comment on whether this technology **offers** promise to clean-up the large crude oil spill in the Persian Gulf. A panel **of** experts drawn from the **ASM's** American Academy **of** Microbiology and Public and Scientific Affairs Board provided the EPA with the following statements:*

Microbial degradation of petroleum is a process naturally occurring in the environment and it can be enhanced. Bioremediation, which employs microbiological processes to effect remediation of environmental damage, shows great promise for enhancing rates of hydrocarbon biodegration. However, claims of rapid or simple solutions of environmental problems by bioremediation cannot be supported by existing evidence from both laboratory and field experiments.

The potential for bioremediation of oil at sea is limited. Of the options available, the application of large quantities of oil-degrading bacteria and nutrients to support their growth can be considered. At the present time, there are no definitive scientific data that unequivocally demonstrates that addition of large quantities of oil-degrading bacteria to oil in the open ocean leads to removal of the oil by biodegradation. Addition of microorganisms to oil in controlled laboratory studies has been shown to enhance biodegradation, but conclusive data from field experiments are not available and will be difficult to obtain. Generally, microbial degradation of oil, even under the most favourable of laboratory conditions, takes weeks to months. The purpose and documented results of the use of bioremediation is to enhance degradation over extended time periods, rather than to achieve short term immediate results.

Data from laboratory studies suggests that addition of microorganisms to the open ocean should not cause significant adverse ecological effects, either directly to sensitive marine species or indirectly to the environment by causing an increase in bacterial biomass or eutrophication. Any product or collection of microorganisms to be applied to a spill, however, must be free of pathogens or toxic chemical constituents.

Addition of oil-degrading bacteria may cause the oil to be emulsified and small droplets formed that can be physically dispersed. Bacterial emulsification in contrast to chemical dispersants does not produce toxic effects. Thus, the environmental concern, if any, should be focussed on ecological effects of dispersed oil. Adverse ecological effects of dispersed oil are possible, but they depend on the type and sensitivity of the exposed environment and would be of most concern for embayments and shallow waters.

In proximity to intakes for drinking water, seeding wjth microorganisms should not be done because dispersed oil will reach these intakes and potentially have public health consequences.

Physical methods for the effective application of oil-degrading bacteria to oil at sea have not been tested, particularly under turbulent conditions frequently encountered in open ocean. Without adequate information regarding field-tested methods for application of bacteria, there is no guarantee that bacteria can be brought into contact with oil long enough to induce significantly enhanced degradation. In protected bays, wetlands, and small estuaries, concerns for how the microorganisms are applied is less important than in the open ocean.

Any benefits accruing from use of oil degrading microorganisms will be for the long term and must be weighed against short term problems. If products are used in the open ocean, carriers causing aggregation and/or sedimentation of the oil should be avoided because of potential adverse impact to the ecology of benthic communities. Bioremediation in the open sea may not prove cost effective, although bioremediaiton of coastal areas has been shown to work and to be cost effective, as well. For long term recovery of the Persian Gulf, bioremediation should be of value. Addition of fertilisers, as well as microorganisms, is an approach to be considered, particularly for near shore and coastal regions.

The benefits of oil bioremediation at sea are at this time indeterminate, and it is likely that natural events will be as effective in removing oil from the sea surface as attempts to accelerate this biological process.

Acknowledgments

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Bioremediation For Oil Spills - Update

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What is bioremediation?

Bioremediation is the enhancement of microbial degradation of oil. By adding fertiliser or other products, this technology attempts to speed up the microbial processes that break down oil. Bioremediation is also used in terrestrial and other applications, for example, for sewage treatment, for contaminated soils, and experimentally for hazardous wastes.

Three main types of bioremediation technologies are currently being developed or applied in oil spill clean-up: addition of fertiliser to oiled shorelines, addition of, microbial products to oiled shorelines, and open water application (as a primary response) of either fertiliser or microbial products to recently spilled oil. Since all of these technologies are attempting to accelerate biodegradation, this update presents a short summary of the processes of oil biodegradation, followed by a discussion of three potential uses of this technology.

How does biodegradation work?

Biodegradation is one of the main ways that spilled oil is weathered. It occurs in most environments, but at varying rates, depending on localised environmental conditions and on the composition of the oil (heavier oils are more resistant to biodegradation than lighter oils) (Atlas 1975). Among the many environmental factors that will affect biodegradation rates, oxygen, nutrients, and temperature are probably the most important (Atlas 1981; DeFlaun and Mayer 1983).

Simply adding oil to an environment will stimulate growth of indigenous microbes, since the oil provides increased amounts of carbon, the microbes' food source (Lee and Levy 1991). Several researchers have documented a lag period before indigenous microbial communities begin to degrade oil (Fusey and Oudot 1984; Westlake and Cook 1980). This may be due to the fact that oil is initially toxic to microbial organisms, and the most toxic fractions must be weathered before microbes can grow, a time period of several days to several weeks (Lee and Levy 1989).

The primary processes of microbial degradation are aerobic (requiring oxygen); 'though anaerobic degradation may occur at very low rates. Low energy, sheltered environments may have the lowest rates of biodegradation, especially in subsurface sediments. Oil in anaerobic sediments in marshes or other environments may degrade very little, with oil persisting in some cases for several years (Atlas 198 1; Lee and Levy 1991). High energy environments usually show rapid biodegradation, in part, because of physical weathering, but also because wave action supplies oxygen and nutrients to the microbial communities. Microbial populations that undergo rapid growth in the presence of spilled oil may become limited by inadequate amounts of nitrogen and/or phosphorus. Field tests on sandy beaches found that fertiliser addition was effective in areas that were heavily oiled, but was not effective for areas that were lightly oiled,

since unassisted biodegradation occurred very rapidly in the latter case (Lee and Levy 1991). Nutrients are less likely to be limiting in the water column for degradation of suspended oil particles, than for oil on shorelines or concentrated in oil slicks (Atlas 1981).

At extremely high salinities, biodegradation is inhibited. At salinities above 33 ppt, degradation rates of petroleum hydrocarbons decreased as salinity increased, up to approximately 200 ppt (Ward and Brock 1978).

Fertiliser addition

The theory behind fertiliser bioremediation is simple: microbes already living on an impacted shoreline have a sudden new source of food: carbon in the spilled oil. After the initial toxicity of the oil wears off, or indigenous microbial populations become acclimatised, all that limits their population growth is lack of nutrients, specifically nitrogen and/or phosphorus. With the addition of nutrients, the microbial population increases, and more oil is degraded, at a faster overall rate than without the fertiliser.

Several studies of fertiliser enhancement on biodegradation of oil by naturally occurring microbes have been conducted in laboratory settings (Atlas 1981; Lee and Levy 1987; Westlake et al. 1978). Many have concluded that fertiliser enhancement has potential as a clean-up tool on oiled shorelines. Field experiments have also been conducted, but these have not always corroborated the laboratory results (Fusey and Oudot 1984, Lee and Levy 1991). Results from field tests are less clear, in part because it is difficult to statistically measure differences in biodegradation rates between control areas and fertilised areas. Some of these difficulties are a result of the high spatial variability in the distribution of oil in sediments of impacted areas.

Several recent studies on fertilisation were conducted in Alaska as a follow up to the Exxon Valdez spill (Prince et al. 1990; Pritchard et al. 1991). Though some measures of oil degradation in these studies were higher in some fertilised plots than in control plots, the overall effectiveness of fertiliser enhancement could not be shown statistically. Despite the mixed results, this study has been cited as proving that bioremediation by fertiliser is an unequivocal success (Pritchard and Costa 1991).

Toxicity

The potential advantages of fertiliser bioremediation must be balanced against possible detrimental environmental effects, including introduction of contaminants, toxicity to aquatic organisms and physical impacts. Some fertiliser products, whose primary use is in a terrestrial setting, may contain trace metals as micro-nutrients (e.g. copper) that would be introduced into an aquatic environment with potentially much more significant toxicological effects (Mearns 1991). Others may produce by-products such as ammonia and/or nitrates that are toxic to aquatic organisms at certain concentrations (U.S. EPA 1989). Intertidal organisms that are directly exposed during application of the undiluted fertiliser solution may be adversely impacted. In addition, physical disturbance from the application process and from monitoring will have some impacts on the shoreline, especially in sensitive environments such as marshes.

Monitoring

Fertiliser use is still experimental in marine environments, therefore any application should include a monitoring program to determine if the desired objectives have been met, and if any adverse effects have been minimised or, are at acceptable levels o' risk. See the following guidelines for microbial products for suggestions on monitoring.

Fertilisers used in bioremediation in Alaska

*Oleophilic fertilisers (such as Inipol **EAP 22)***

Oleophilic fertilisers such as Inipol are used because they are "sticky" and adhere to oil on rocks or other substrates. In theory, these fertilisers stay at the oil-water interface, and are therefore readily accessible to microbes. Inipol contains oleic acid (a source of carbon), urea (a source of nitrogen), tri (laureth-4)-phosphate, and 2-butoxy- ethanol (Pritchard et al. 1991). Since addition of oil alone will stimulate bacterial growth, this complicates the evaluation of the effectiveness of oleophilic fertilisers such as Inipol. Do these products appear to work better because the microbes are eating the carbon in the Inipol? Or are the microbes actually eating more of the spilled oil?

The disadvantages of Inipol are that its components are toxic, including 2- butoxy-ethanol, and urea, which produces ammonia when it comes in contact with water. 2-butoxy-ethanol is toxic to mammals, especially in the first 48 hours after application. Also, special safety precautions need to be enacted for workers who handle Inipol.

Inorganic fertilisers

These include a variety of water soluble mixes of nitrogen and phosphorus, mixed with seawater and sprayed on beaches. Advantages are that these chemicals are readily available, inexpensive, and usually made up of compounds with well known properties. Disadvantages include possible toxicity from direct impacts to plants or animals in intertidal zone, and the possibility that fertiliser will be washed away with the tides.

Inorganic fertiliser by sprinkler application

This was an experimental technology tried in Alaska with some promising results, although without proper control plots. Inorganic fertiliser was mixed with seawater and sprayed through an offshore pump and sprinkler system over the intertidal zone. Sprinklers were on for 4 hours at a time, every four days '(Winter 1991). The potential advantages of this system are that nutrients as well as oxygen may bettei reach subtidal sediments, and that concentrations of fertiliser are controlled. Disadvantages are that the system is elaborate to set up, and has only been tried once.

Slow release capsules (Customblen pellets)

This particular slow-release product contains ammonium nitrate, calcium phosphate and ammonium phosphates (with a nitrogen to phosphorus ratio of 28:8) encased in a polymerised vegetable oil (Prince et al. 1990). Capsules lodge in between rocks and in crevices in the intertidal zone, and release nutrients slowly over time. Advantages are that the dosage is controlled at low levels and that pellets may work their way down into sediments, providing fertiliser to the subsurface. Providing pellets remain in the intertidal zone, Customblen may not need to be applied as frequently as liquid fertilisers. Disadvantages are that pellets may wash away or lodge at the high tide zone on beaches with strong wave action. Concentrations of pellets higher than the recommended application could collect in one location and create concentrations of ammonia that are toxic to aquatic organisms.

Summary *- fertiliser*

Application of fertiliser as a treatment for oiled shorelines is complicated, because natural biodegradation rates vary considerably depending on the environment in question. Temperature is an important consideration, as is the amount of organic matter and nutrients, salinity, and oxygen. Like most other treatment technologies, decisions will probably need to be made on a case by case basis. What works in one situation, may not be effective or may be inappropriate for another situation. Monitoring should be conducted to verify the effectiveness of the application, and to document any adverse impacts that may occur.

Fertiliser may be most appropriately considered in the following situations:

- Sheltered shorelines that are heavily oiled;
- Shorelines with substantial subsurface oil that may degrade very slowly. (In this case, increased oxygen may need to be supplied to the subsurface);
- Sensitive environments that will be adversely impacted by other clean-up methods, especially marshes and wetlands.

Microbial products

We have the least information on microbial products, since little research on the effectiveness of specific products has been conducted to date. Yet we get the most requests for information on microbial products, since the vendors of these products are extremely active in marketing and self-promotion.

The effectiveness of adding microbes to the environment to enhance biodegradation is not well supported in the scientific literature (Atlas 198 1). In fact, studies indicate that addition of microbes to an open environment probably does not increase biodegradation, because “foreign” strains of bacteria disappear quickly from the microbial community, since they are out-competed by indigenous species (Lee and Levy 1989). Also, no strain of bacteria, whether indigenous or from a product application, is likely to actively degrade oil until after the most toxic components of the oil have evaporated (Lee and Levy 1987). Therefore, claims of “instant success” from microbial products should be regarded with scepticism.

Currently, there are few objective and scientific studies that have tested microbial products currently on the market The most comprehensive was conducted by Venosa et al. (1991a, 1991 b) of the EPA Cincinnati Lab. The results of these studies were discussed briefly at our Santa Barbara meeting. In brief, the lab study compared 11 microbial products'with fertiliser alone at 15°C for biodegradation-of weathered Prudhoe Bay. crude oil. Two products showed a statistically significant increase in biodegradation over fertiliser. However, these products performed as well when steriiised (dead microbes) as with live microbes. Both of the two highest performers were then tested in a controlled, replicated field test in Alaska. In the field, no significant difference in biodegradation could be detected between the control plots, the fertilised plots, or the plots treated with microbial products. One additional study, presently in draft form, is the monitoring program done at Seal Beach in California (Pritchard 1991-Draft). Preliminary results indicate that the microbial product applied did not increase biodegradation compared with the control sample.

Venosa's lab study (1991a) is available in the Oil Spill Conference Proceedings, and his field study (1991 b) is in press. The field portion of the study provides a good example of a controlled and replicated field study, and you may want to look at it for that purpose.

Product evaluation

A three-tiered protocol is being developed by EPA for standardised testing and evaluation of microbial products. This will include preliminary screening, laboratory testing (including toxicity testing), tests using microcosms, and finally, field testing. These protocols are still being developed and are expected to be in place in l-2 years.

In the meantime, it is up to us and any other potential user to ***evaluate the products individually.*** This is time consuming and inefficient, but at this time, we cannot assume that ***any bioremediation product on the market has undergone a standardised screening process.*** It also means that you are dependent on the vendor to supply you with information on their product. The following considerations are guidelines that may help in evaluating a microbial or other bioremediation product:

Initial Screening

Consider factors such as the reputation of the vendor, product safety and special handling requirements, presence of harmful or pathogenic ingredients, especially known human or animal pathogens such as ***Vibrio sp.,*** or *Klebsiella* ***sp.***

If possible, verify claims made by vendors. We have encountered several instances where false claims were made that certain products were approved or recommended for use by governmental agencies. Follow-up inquiries indicated that there was no factual basis for such statements.

Toxicity

Consider the potential toxic properties of the "binder" chemicals, including possible trace elements in fertiliser components. Companies do not like to reveal the exact composition of their products, claiming that it is proprietary information. However, if

they claim that there are "no toxic elements" in their product, you *should ask **for proof of** this* (results of toxicity tests, or full disclosure of the composition of the product).

Ask for the results of aquatic bioassays or other toxicity tests that the company has performed. Expect as a minimum at least one standard bioassay for acute effects performed on recognised sensitive species (see EPA guidelines). Chronic toxicity tests would also be useful, as well as tests performed on more than one species. (If the company has not done toxicity testing, you may wish to require that this be performed as part of the monitoring program).

Effectiveness

Bioremediation is still an evolving technology, so I would consider any application as an experimental one. The bottom line is whether the product promotes the conversion of petroleum hydrocarbon components into other, less toxic compounds. As a minimum, companies should be able to prove (from chemical lab results) that their products biodegrade the oil of interest or a very similar one at rates substantially greater than fertiliser alone, in a controlled laboratory setting. *This does not, however, guarantee that the product will perform in the field.*

Many products are "tailored" for certain environments, such as a specific temperature range, or for use with certain types of oil. Ask the company to show you the results of their tests showing that the product is effective *under the conditions **of** the proposed application.* For instance, product Z is designed to be used in sewage treatment plants at temperatures of 25°C, but you are considering application in Alaska (15°C) on weathered crude oil. The effectiveness data from the company's tests may not translate to the different environment.

Monitoring

Since it is very unusual to have pre-existing data on performance of a product in the field, a monitoring program should be set up with any bioremediation application to determine if the product is working as intended. The monitoring process is likely to cost more than the application of the product, and should be considered when deciding whether to use a microbial product.

Monitoring should be well planned and include carefully selected control sites with replicate sampling of all test and control plots. Select in advance the endpoints to be measured to determine if biodegradation is occurring at accelerated rates, such as total hydrocarbons or total alkanes. Avoid the use of qualitative measures such as "visual differences", which are very difficult to interpret. Select an appropriate time frame for collecting samples, preferably including before, soon after application, and several samples over a longer time period.

Summary - microbial products

The decision on whether to use microbial products should be made only after careful evaluation of the products available, and after evaluating data on their toxicity and effectiveness. Some states are beginning to set, up screening criteria for products that is similar to the EPA protocols. This approach should rule out consideration of any new, unknown product during a spill. Without reliable data on the effectiveness and potential toxicity of a microbial product, it is impossible to'make an informed decision on its application in the marine environment.

Open Water Use

Studies from the early 1970s in laboratory and simulated large tank situations have investigated the use of addition of fertiliser on open water oil slicks (Atlas and Bartha 1973). However, I know of no studies where open water use of bioremediation (microbial or fertiliser) has been scientifically evaluated in an open ocean situation. Many questions remain about the potential for this type of application, including the following:

* Would bioremediation be effective on a recently spilled open water oil slick?
* How does natural biodegradation occur on the water surface?
* What is the lag time before microbial action begins to degrade oil on the water surface? (Lag times have been found of 3-5 days for lab tests, and 10-l 1 days in field tests).
* Could a microbial product or fertiliser stay with the oil as the slick moves?
* Do bioremediation products applied on open water actually act as dispersants or surfactants, and redistribute oil into the water column? (If this is the case, are they in fact, dispersants masquerading under a different name?)

Summary - open water bioremediation

The long history of attempting to document the effectiveness of dispersants is very applicable to open water bioremediation techniques. Controlled field studies under real oil spill conditions are extremely difficult to conduct, and research on intentionally spilled oil even more so. Because of the difficulties in verifying effectiveness in open water, this is presently the least promising application of bioremediation technology. We feel that open water use of bioremediation is highly experimental, and that many substantial questions still need to be answered before this technology should be considered. At this time, we recommend that you stay with other known and tested methods for primary response on open water.

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