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**Subject: Research into vegetable oil based biodiesels as a  
cleaning agent for heavy oil spills**

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## **1 Executive Summary**

AMSA commissioned the Centre for Marine Studies of The University of Queensland, through UniQuest, to investigate the use of biodiesels to clean selected substances following a marine oil spill. This research was limited to a literature review of this use of biodiesels and a limited battery of laboratory tests. In short, this report addresses key questions regarding the efficacy of using biodiesel to clean up marine oil spills, particularly spills occurring in Australian conditions.

The literature review addressed the following questions:

- Are biodiesels effective in cleaning oil spills;
- What biodiesels have been evaluated for this purpose and what were the results;
- What properties have been identified that confer effectiveness in oil spill cleaning;
- Are there any deleterious properties of biodiesel in the marine environment;
- What biodiesels are available in Australia?

The review revealed that while research into biodiesels as oil cleaning agents commenced more than a decade ago, few definitive conclusions about its relative merits as a cleaning agent have resulted. A limited range of biodiesels have been evaluated, chiefly canola and soy-based products, with one experiment using a biodiesel derived from palm oil and none using coconut oil. The majority of experiments have been small scale laboratory experiments with limited application in an actual oil spill response. Only a single paper reports results of the experimental application of biodiesel to an actual spill. However, despite these limitations, the available literature suggests that biodiesels offer effective solvent-based cleaning action in combination with greatly reduced toxicity to marine environments. They clearly warrant further research.

From our survey of potential biodiesel cleaning agents those derived from palm oil and coconut oil were judged to possess favourable physical and chemical properties, and to be readily available in Australia. Thus they were selected for trial in a laboratory experiment as the candidate biodiesels for evaluation against better known agents used in oil spill clean up.

The laboratory experiment was designed to:

- Measure the effectiveness with which the biodiesels removed oil from a variety of natural and man-made substrates;
- Compare the effectiveness of candidate biodiesels to that of commercially available and commonly used oil spill cleaning products;
- Provide recommendations as to what further research may be required in order to make biodiesel a viable candidate for oil spill remediation.

Our results supported previous findings that biodiesel is an effective cleaner of spilled oil. When applied to a range of hard natural and synthetic substrates covered in bunker oil, biodiesel-based agents were at least as effective as other reference cleaners. Oil was removed efficiently from aluminium, fibreglass, granite, coral and concrete far in excess of the rates achieved by the application of seawater (control) alone. However removal rates tended to be lower and more variable for soft substrates such as mud and sand. This may indicate that the oil-biodiesel mixture becomes bound to the substrate reducing its uptake into the water fraction and subsequent recovery by hexane extraction. Due to the difficulty of replicating field conditions in laboratory experiments with soft substrates (e.g., the presence of biofilms and the nature of fine sediment saturation, which might reduce the tendency of oil-biodiesel mixtures to migrate into or bind with a sediment) we strongly recommend *in situ* field studies to investigate the use of biodiesels for cleaning habitats with soft sediments.

Overall the experiment revealed little to distinguish between the performances of the two biodiesels, i.e. palm oil and coconut oil. However, while the choice between these for use in large quantities will involve issues such as price, shelf life and toxicity, further comparative tests on them are recommended. Additionally, further research is recommended to assess the net environmental benefit of biodiesel application in the event of an oil spill in the Australian marine environment because our laboratory experiments did not cover this aspect. Of particular concern are the following:

- Ecotoxicity to critical habitats and their associated organisms, such as mangroves, coral reefs and seagrass;
- Formulating appropriate application techniques for field work/oil spill response; and,
- How application, along with toxicity and the resultant oil-biodiesel mixture, might impact the marine environment;

Once these aspects are understood, funding for field work trials are recommended to evaluate the effectiveness of biodiesel in a large scale application and the proposed application methods. Biodiesel likely has an important role in oil spill response to reduce adhesion to objects, break up the oil and increase the biodegradability of the oil. Only extensive testing and research over several years will reveal the full extent of this role.

## 2 Literature Review

### 2.1 Introduction

The structure of this literature review differs in structure from the original tender because in the survey of publications it became apparent that some sections offered in the original proposal would be devoid of content, as published literature on some aspects is lacking, and other topics required additional headings or rearrangement to provide a logical structure to the literature review. The table presented in Appendix C provides a summary of the location of information foreshadowed in the original proposal to AMSA annotated with comments about the location of listed information in this report. The following literature review provides a logically structured exposition of the current status of our knowledge about biodiesels themselves and empirical knowledge about their potential as a cleaning agent for use in oil spills.

#### 2.1.1 Biodiesel

Biodiesel is produced from a wide range of oil seed crops, including but not limited to: corn, soybean, rapeseed/Canola, sunflower, jatropha, coconut and palm kernel [1]. The oil from these seeds is pressed and extracted, then subjected to one of a variety of processes, the most common of which is known as transesterification [2]. Through transesterification, the vegetable oil is reacted with an alcohol, usually methanol, and a basic catalyser, often sodium hydroxide (NaOH) or potassium hydroxide (KOH) [3]. The reagents used, as well as the time and temperature of the reaction, influence the yield and the optimal reaction conditions are specific to each type of oil. Thorough treatment of biodiesel production can be found in reviews by Ma et al. [2]; Canakci & Van Gerpen [4]; and Fukuda et al. [5].

While the extant literature indicates that biodiesel is a promising agent for the cleanup of oil spills, its efficacy is far from conclusive. Among the publications on its use as a treatment for oil spills, there is an obvious over-reliance on laboratory data, admittedly tempered by frequent exhortations to work in the field rather than the laboratory. While being mindful of over-reliance of laboratory data and the potential limitations to extrapolating laboratory experiment results to the field, the data are indicative that biodiesel should be carefully considered in contemporary oil spill response strategies and, more importantly, further tested in more realistic experiments.

## **2.1.2 Properties of biodiesel**

A number of properties of biodiesel need to be considered in assessing whether or not it can, or should, be used in oil spill response and these will be separately discussed.

### **2.1.2.1 Miscibility**

The capacity of biodiesel as a solvent for heavy oils is not surprising, as the most common usage of biodiesels is as an additive to petroleum derived diesel, thus its miscibility with petroleum distillate products has been well established [2, 6, 7]. Tests measuring canola, corn, soybean and sunflower biodiesels indicate that they are all strong solvents [8], confirming and quantifying the ability of biodiesels to act as a hydrocarbon solvent. Furthermore, biodiesel has been shown to act as a strong solvent to coal tar, a particularly recalcitrant hydrocarbon mixture [9]. The importance of this strong solvent behaviour was shown in the field trials by Fernández-Álvarez et al. [10, 11]. Heavy fuel oil that had been thoroughly weathered in the aftermath of the *Prestige* oil spill (see §2.2.7 and §2.2.9 for detailed outline of these studies) presented as an asphalt and resin layer on rocks that was resistant to degradation. Biodiesel applied to this tarry surface was incorporated rapidly into the oil layer, without which further attempts at removal would have been futile.

### **2.1.2.2 Viscosity and buoyancy**

In addition to its solvent properties, biodiesel has physical properties that suggest efficacy as a cleansing agent for oil spills. Kinematic viscosity is an important characteristic of biodiesel in its application as an oil spill remediation agent. Because biodiesel has been widely studied as an alternative fuel, its viscosity has been thoroughly examined in order to predict its performance in engines. These studies indicate that transesterification results in a significant decrease in the viscosity of the resultant methyl esters, by up to an order of magnitude, compared to the vegetable oil from which they are made [12-14]. The viscosity is further dependent upon the relative abundance of particular fatty acid methyl esters, and the greater the proportion of short chain fatty acids the lower the viscosity [15].

Biodiesels generally have viscosities that are comparable to low sulphur (No. 2) diesel fuel [12, 15] and are much less viscous than heavy fuel oils [16]. The much lower viscosity of biodiesel, along with its solvent properties, suggests that when biodiesel is used to dissolve spilled oils, the resultant biodiesel-oil mixture will be

much less viscous than the oil alone, facilitating its removal from intertidal structures. However, research indicates that the viscosity of oil-biodiesel mixtures is not so low as to result in significant penetration into sandy sediments [17].

### **2.1.2.3 Biodegradability**

One of the most significant limitations on biodiesel is its tendency to rapidly biodegrade, resulting in a limited shelf life for stored, neat biodiesel (i.e. pure) and biodiesel-diesel mixtures (A. Cowan, Natural Fuels Ltd, Darwin – personal communication). In fact, biodiesel is considered readily biodegradable by USEPA standards [18]. Whereas this high degree of biodegradation is a limitation for the fuel industry, it is a great benefit to oil spill bioremediation. When used as a solvent, a high degree of biodegradability is conferred on the mixture; depending upon the type of oil, the type of biodiesel and the ratio of oil to biodiesel [19]. This high degree of biodegradability of both biodiesel and biodiesel-oil mixtures results from the bioavailability<sup>1</sup> of biodiesel and the process of co-metabolism<sup>2</sup> [9, 20, 21].

Biodiesel degrades faster and to a greater degree than petroleum derived fuels, particularly heavy oils. One study found that when exposed to the elements over the course of 28 days, biodiesel degradation reached nearly 100%, whereas gasoline and diesel fuel degraded 56% and 50%, respectively. The final level of degradation of mixtures of the mineral fuels and biodiesel was relative to its biodiesel content (i.e. a greater proportion of biodiesel resulted in a greater degree of biodegradation) [20]. Another study found that the half-life of biodiesel in seawater was approximately four days [22]. Through these comparisons to mineral diesels and other petroleum-derived fuels, as well as the previously discussed laboratory and field experiments, the biodegradability of biodiesel has been thoroughly established. Biodegradability can be enhanced or inhibited by abiotic conditions and the level of bacteria present, but in all conditions to date, biodiesel has shown to be at least as degradable as the most readily degradable petroleum components.

### **2.1.2.4 Toxicity**

The earliest toxicity test of biodiesel was an evaluation of its phytotoxicity<sup>3</sup>, its harm to plant growth [23]. This study found that with all plants tested, biodiesel was at

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<sup>1</sup> The ease with which it can be used by living organisms

<sup>2</sup> Degradation brought about specifically by microbes

<sup>3</sup> Toxicity to plants

worst, as toxic to the plant as marine diesel, however, in most cases, biodiesel was significantly less toxic and less persistent than diesel. Similar results were reported for the effects on three species of freshwater invertebrate as well as one species of freshwater fish [24].

In a soil toxicity study, corn *Zea mays* and soybean *Glycine max* were grown in soil contaminated with biodiesel, diesel or blends of the two. Both plants grew more quickly and generated greater biomass when planted in the biodiesel soil, indicating that biodiesel was either less harmful to the plants or was degraded more rapidly in the soil [24]. Another soil study found that biodiesel degradation in aerobic soils may produce toxic intermediate products and that these short-lived products showed deleterious effects to the amphipod *Hyalella azteca* and inhibited the soil bacterium *Vibrio fischeri* [25]. However, these effects were reduced significantly within two weeks and eliminated entirely by eight weeks [25].

Subsequent tests indicate that a variety of biodiesels are considerably less toxic than reference fuels such as diesel and 2-D low sulphur diesel in a variety of animals, including rats, rabbits, the waterflea *Daphnia magna*, the rainbow trout *Oncorhynchus mykiss* and soil bacteria [26, 27]. As an often cited example, the toxicity of rapeseed methyl ester to *D. magna* was found to be 23 mg.L<sup>-1</sup>, where as the toxicity of table salt was found to be 3.7 mg.L<sup>-1</sup>, more than six fold more toxic [26]. Furthermore, when blends of mineral diesel and biodiesel were compared, toxicity was inversely proportional to the percentage of biodiesel in the mixture [27].

Eco-toxicological studies using mammals, fish, daphnia, algae and bacteria found that rapeseed methyl ester and soybean methyl ester were dramatically less toxic than diesel fuel to all of the organisms except mammals, which were equal [28, 29]. Additionally, biodiesel-diesel blends, despite showing lower toxicity than diesel, still resulted in negative effects on *O. mykiss* fry, such as fin erosion and curling of the body after high exposure [29].

While further understanding of the deleterious effects biodiesel may have on the environment is required, the toxicological studies to date indicate that biodiesel can be toxic, but in many cases much less toxic to a wide range of aquatic and soil organisms than the spilled oil it is used to clean. The toxicity of a cleaning agent

must be well understood in a wide variety of conditions, environments and test organisms in order to ensure a net environmental benefit.

### **2.1.2.5 Biodiesel production and availability in Australia**

#### **2.1.2.5.1 Current production and availability**

The Australian biodiesel industry is considered to still be in its infancy [7], and this is reflected in the relatively limited availability of biodiesel and a narrow range of production sources. The primary oil source for biodiesel production in Australia is currently animal tallow and waste cooking oil [7]. Figure 1 depicts the major producers of biodiesel in Australia, the oil used to produce biodiesel and the production capacity per year. Recent measures by the government have encouraged the growth of biodiesel production and a target of 350 million litres produced per year was scheduled by 2010 [30]. This target had been achieved by 2006 [31], reflecting a rapid increase in production in a relatively short period of time. Though the increase in production is promising, the lack of diversification of the oils used to make biodiesel has significant impacts on the choices of biodiesel in the event of an oil spill. Waste cooking oil biodiesel has been shown to be less effective at cleaning oil spills than either soybean and rapeseed biodiesel [19], and while no studies evaluating biodiesel produced from tallow have been done, the relatively high proportion of long chain fatty acids result in a particularly high melting point, limiting its suitability in temperatures near or below 15°C [2].

In the case of a large oil spill, a large quantity of biodiesel would be needed in a very short period of time, thus limiting the choice of biodiesels to those that are available nationally or in the Australasian region. Considering the relative unsuitability of tallow, the Australian biodiesel market offers one potentially promising biodiesel, palm oil biodiesel. Approximately 160 million litres of palm oil biodiesel are currently produced per year domestically (mainly from imported materials). Coconut biodiesel is available from the Philippines, where a government initiative has stimulated the biofuel industry. Both of these biodiesels are promising candidates for oil spill remediation.

Palm oil biodiesel has a viscosity similar to that of canola and soybean, which have shown to be effective cleaners. Further, palm oil biodiesel has a very high proportion of palmitic acid [15], which has been shown to significantly stimulate biodegradation of oil [32]. Coconut biodiesel may be even more effective than palm oil, as it is

composed primarily of the short chain fatty acids lauric and myristic acid. In fact, coconut biodiesel has the greatest proportion of these two fatty acids when compared to 14 of the most common biodiesels [15]. Though lauric acid was not tested, myristic acid was found by Obbard et al. [32] to enhance the effectiveness of biodegradation greater than any of the other fatty acids they tested. Another potentially attractive property of coconut biodiesel is its very low viscosity [15], which may allow for better penetration of sediments with low pore size, where other biodiesels have shown a limited capacity [17, 19], thus allowing for treatment of substrates into which spilled oil has penetrated.

Both oil palm and coconut palm oil have high levels of production. Both are amongst the leading oil producing plants per hectare, as oil palms and coconut palms are capable of producing up to 5950 and 2689 litres of oil per hectare respectively, whereas canola and rapeseed are only capable of producing up to 1190 litres per hectare and soybeans only 446 litres per hectare [33].

Drawbacks to the use of coconut and palm oil biodiesels include the price of importing the source materials, the crude oils or the refined product and the ecological consequences of deforestation in the countries that grow oil palms or coconut palms in monoculture plantations [34]. Deforestation can also result in eutrophication of the marine environment due to soil erosion and nutrient runoff [35]. Recent laws aimed at decreasing the importation of fossil fuels into the Philippines have resulted in predicted use of B5 coconut biodiesel (5% biodiesel, 95% fossil diesel) by 2008 and B10 by 2010. This will likely decrease the amount of coconut oil available for export to Australia. It will also increase the price while encouraging an increase in coconut production at the expense of native forests. For its part, palm oil is increasing in demand for biodiesel as well as for use as a food oil worldwide [36].

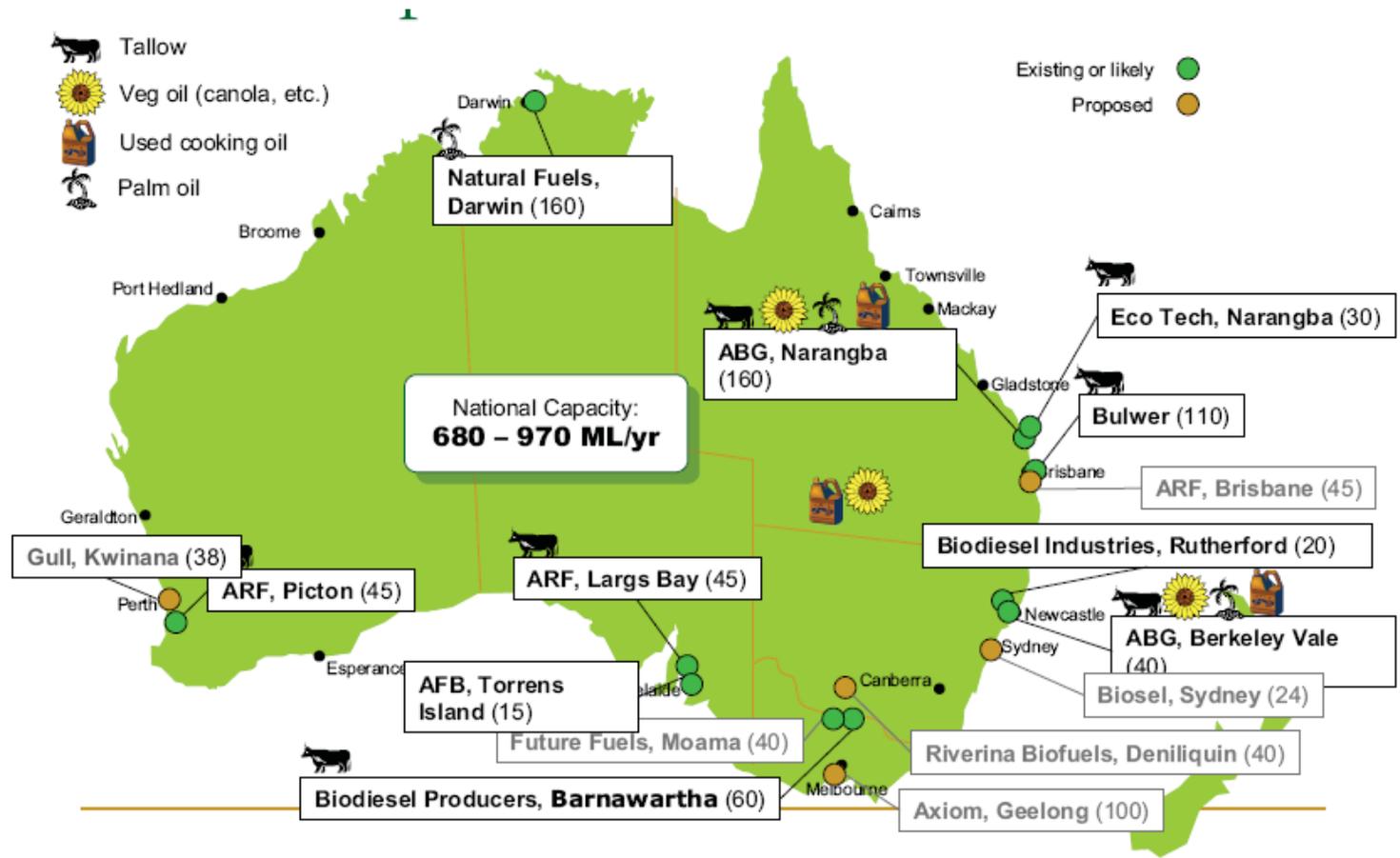


Figure 1. Biodiesel production sources in capacity in Australia as of August 2007. Image courtesy of Australian Farmers Fuel Pty Ltd (Enffue).

Note: Some source oils are proposed (i.e. ABG, Narangba currently only produces biodiesel with tallow and used cooking oil).

#### **2.1.2.5.2 Likely near future supply and availability**

Further expansion of the Australian biodiesel industry would be greatly beneficial to the national interest regardless of its application. In terms of oil spill bioremediation, a diverse assortment of biodiesels available may be beneficial for use in different situations. For example, in a shoreline oil spill, different biodiesels can be applied to different substrates. A biodiesel with less viscosity may be applied to substrates with a high surface area to volume ratio, such as coral rubble, as the lower viscosity may enable more effective cleaning, whereas a higher viscosity biodiesel can be applied to low surface area to volume substrates, like large boulders, in order to maximise the contact time between the biodiesel and adherent oil.

Additionally, the maturation of the biodiesel industry may result in the production of biodiesels from novel sources. One of the most promising oil sources currently being researched is microalgae. Algae have the potential to produce between 58,700-136,900 litres per hectare, nearly 10-23 times greater than that of the next highest producing oil source, the oil palm [33]. Additionally, microalgae are capable of doubling biomass within 24 hours, a growth rate well beyond terrestrial oil producing plants. Many microalgae use carbon dioxide as their primary carbon source. Growing algae to act as a sink for carbon dioxide present in the exhaust from fossil fuel power plants and to clean chemical wastewater from factories [37, 38]. The production of oil as a by product of such systems has obvious merits. Biodiesel produced from algal oil has yet to be produced in any appreciable quantity, but several firms throughout Australasia are investigating this process.

## **2.2 Trials of biodiesel in oil spill remediation**

The following section presents a description and critique of the published research on the use of biodiesel in oil spill cleanup.

### **2.2.1 Miller & Mudge, 1997**

The initial proponents for the utilisation of biodiesel as a cleaner for heavy oil spills appear to be Miller & Mudge [17] in 1997, who also cite unpublished data from von Wedel. Their study represents the first research into the use of biodiesel as a cleaning agent for oil spills. The authors contaminated sand samples with light crude oil floating on seawater. The sand was contained in a box fitted with plastic tubing to facilitate water flow through. Biodiesel was applied to the floating oil slick, which was

then stranded on the sand surface as the water proceeded to flow through the sand. Surface samples of the sand were taken at intervals over the course of 14 days, and seawater was flushed through the system after each sediment sample was taken. Both the sediment samples and water samples were subjected to hydrocarbon extraction and the recovered samples were further analysed for the hydrocarbon compound quantification to evaluate the ratio of more volatile compounds to more persistent compounds.

Analysing the oiled sand control samples allowed Miller and Mudge to establish a baseline of oil weathering on the sand columns, which indicated that after 14 days shorter chain hydrocarbon compounds were degraded to a high degree than higher chain compounds. Complete degradation occurred in only the shortest chain molecules, which was a factor of their higher volatility. However, due to their low viscosity, these more volatile short chain hydrocarbons also penetrated into deeper layers of the sand column where degradation may be retarded by anaerobic conditions which are deleterious to the hydrocarbon digesting microbes, resulting in enhanced persistence [3]. Longer chain compounds were not found to penetrate deeply into the sand columns; rather, these more viscous compounds were stranded at the surface of the sand.

This study was the first set of baseline data for understanding how biodiesel would behave on a simulated beach substrate. Similar to the crude oil controls, the biodiesel controls showed a variable degree of mobility through the sand, with shorter chain fatty acid methyl esters penetrating more than longer chain molecules. However, only a low proportion of these fatty acids were transported to the deeper portions of the sand columns, and, despite penetrating into the sediment, the biodegradability of the unsaturated fractions appeared to be relatively unaffected.

Analysis of biodiesel treatments indicated that biodiesel application increased the mobility of the crude oil. The more soluble components, short carbon chain molecules were readily dissolved and some of the more resistant components dissolved after longer exposure. Much of this dissolved oil in biodiesel absorbed into the sediment, where it was flushed through with water. This has implications for the biodegradability of the mixture, as microbial break down may be slowed or inhibited at greater depths, whereas in an experiment, the oil-biodiesel mixture can be flushed through and recovered. However, this increased mobility also suggests that water

flushing immediately after biodiesel application may refloat the oil, allowing for recovery and the prevention of sediment penetration.

Perhaps the most important finding of this study was the indication that the application of biodiesel reduced the persistence of oil, even when the mixture penetrated the sediment. However, the results indicated that the later the biodiesel application occurred after the oil contamination occurred, the less positive impact the application had. The authors suggest that this indicates a narrow window of opportunity for treatment with biodiesel and that application must occur immediately after a spill for maximum effectiveness.

While the overall results were favourable in suggesting that biodiesel has a positive impact on both the physical removal and eventual microbial biodegradation of oil spilled on sand, the authors stressed that more research is needed, particularly to determine the optimum application rate of biodiesel, the window of opportunity to provide the maximum benefit and what effects confounding variables, which have been eliminated in laboratory settings, will have on the practical application of biodiesel.

### **2.2.2 Mudge & Pereira, 1999**

In 1999, Mudge and Pereira [39] presented a preliminary report of experiments using rapeseed biodiesel and soy biodiesel and two experimental methods, in this case, batch experiments and box experiments, to evaluate the efficacy of oil removal. The batch method combined a homogenised sample of sediment contaminated with crude oil, which had been weathered for four weeks, with varying volumes of the two biodiesels. These mixtures were agitated to allow sufficient contact between the oil and the biodiesel and then the mobilised hydrocarbon fraction was extracted and the amount of oil removed was quantified. The box experiments determined the amount of crude oil removed by varying volumes of the two biodiesels from boxes of sand by analysing water flushed through the system, similar to the previous work by Miller and Mudge [17].

Biodiesel was found to be effective at mobilising oil from sand, as the batch experiments resulted in nearly complete removal of oil with very little variation resulting from the amount of biodiesel used. In the box experiments, greater volumes of biodiesel resulted in greater oil recovered, and similar to the experiments

of Miller and Mudge [17], application of biodiesel immediately after oil contamination resulted in greater recovery. However, adding greater volumes of biodiesel ameliorated, to a degree, the decreasing efficiency of oil removal when there was delayed application of biodiesel.

It is not surprising that in such a preliminary study, the data were far from conclusive as to what effect the biodiesel was having. There were no statistical evaluations, and much of the results and discussion sections dealt with qualitative observations and speculation. However, as rudimentary as the results were, they do show that biodiesel has some capacity to facilitate oil removal, as comparisons to controls showed favourable results for biodiesel treatments. Similarly, comparisons to control samples indicated that the narrow window of opportunity for biodiesel application may be at least partially alleviated through a greater application rate.

One intriguing observation made by the authors was that a discolouration of seawater flushed through the box experiments was likely to be caused by hydrocarbon-metabolising bacteria. The discolouration was suggested to be metabolic by-products as they showed no spectrometric fluorescence at known hydrocarbon wavelengths. The significance of this finding is that no microbial populations or additional nutrients were added to the systems, thus showing that any microbial biodegradation was due to native microbe populations acting in response to the oil.

### **2.2.3 von Wedel, 2000**

In a 2000 technical research note, von Wedel [22] described a proprietary product called CytoSol, which was described as a vegetable oil methyl ester-based biosolvent with nutrient additives for use in cleaning oil spills. CytoSol cleans in a two step process, the first of which is physical removal through an increase in cohesion and a decrease in viscosity of the oil resulting from its mixture with biodiesel. This step, combined with low-pressure ambient temperature washing, was suggested to remove 50-98% of oil from various shorelines, which can then be recovered.

The second stage of CytoSol cleaning is the biodegradation of any remaining oil, which is enhanced by the nutrients contained in the product which stimulate the native hydrocarbon degrading bacteria. In one study, after CytoSol treatment oil-

contaminated sand was treated with nutrients and tilled for oxygenation, it resulted in a 90% decrease in hydrocarbons after six weeks of treatment.

The research note also refers to biodegradation studies which indicate that CytoSol had a half-life of approximately 4 days in 17°C water. Toxicity studies on *Mysidopsis bahia* shrimp larvae, *Menidia beryllina* fish larvae and *Halitotis refescens* abalone larvae found CytoSol to be 15-20 times less toxic than the reference fuel oil. Application of CytoSol in creek beds or on epifaunal encrusted pilings showed no major disruptions to the environment.

It must be noted that the results from this technical note do not come with any data or statistical analysis. Rather, they are presented simply as “proof” of CytoSol’s efficacy, resulting in a document that appears akin to a press release, rather than a scientific evaluation of this product. Similarly, no data are provided that compare CytoSol to biodiesel that has not been amended with “biodegradation enhancers,” which could be indicative of the usefulness of nutrient fertilising. Overall this Note should be treated with some reservations.

#### **2.2.4 Taylor & Jones, 2001**

The effectiveness of biodiesel in cleaning spills of coal tar, a by-product of coke and gas production, was evaluated by Taylor and Jones [9]. In this study, the biodegradation of coal tar was measured in both laboratory and field conditions by comparing the depletion of polycyclic aromatic hydrocarbon (PAH) compounds by a rapeseed biodiesel and mineral diesel. PAHs are hydrocarbon molecules with multiple carbon rings, which tend to provide stability and resist break down. Additionally, many PAHs are toxic or carcinogenic.

The authors found that coal tar was more soluble in biodiesel than in diesel and that degradation of the lower molecular weight, two- and three-ring PAH molecules, occurred in both diesels, whereas the higher molecular weight molecules with three rings or more were relatively unaffected. Nutrient addition was found to have a negligible effect. Mineral diesel treated samples were found to result in the greatest decrease in PAHs, indicating it was more effective than biodiesel in this study. The authors warn that despite these results, diesel fuel is not a reasonable option for PAH remediation, as it is similar in toxicity to the PAH molecules that are to be removed and other research indicates that diesel fuel is more toxic than crude oil [40].

Furthermore, the authors suggest that the decreased biodegradation rate of the biodiesel treatment may be due to a negative feedback loop resulting from a biodegradation product, methanol, which inhibits further bacterial growth, and that reduction of the amount of biodiesel applied may ameliorate this issue.

### **2.2.5 Pereira & Mudge, 2004**

Pereira and Mudge [19] expanded upon the earlier preliminary report, and further evaluated the efficiency of extraction of light crude oil by rapeseed, soybean and waste cooking oil biodiesels in three sets of experiments: batch, microcosm and mesocosm.

The batch experiments, similar to their previous batch experiment [39], involved applying oil to sand contained in boxes, weathering it for 28 days, homogenising the oil and sand mixture and taking samples presumed to be homogenous. These samples were then treated with varying volumes (30, 40, 50 and 75 ml) of rapeseed, soybean and waste cooking oil biodiesels, and the biodiesel-oil mixture was extracted and the extracted hydrocarbons quantified. Controls indicated that water application resulted in the physical removal of 15% of the crude oil. Rapeseed biodiesel was the most effective of the three biodiesels tested, removing 90%-96.5% of the crude oil depending upon the volume of biodiesel applied. Rapeseed biodiesel was more effective than either of the other biodiesels at each respective volume. When larger volumes were used the degree of oil removal by all three biodiesels was significantly greater. For example, the removal rate of 96.5% resulting from the application of 75 ml of biodiesel was significantly greater than the 90% of crude oil removed by the 30 ml application. Soybean biodiesel approached the removal efficiency of rapeseed biodiesel at larger volumes, whereas waste cooking oil was much less effective than either of the other two at all volumes.

Microcosm experiments were used to determine the amount of oil removed from an oil contaminated box of sand, similar to previous studies [17, 39]. Measurements were made at the sand surface and also of the water flushed through the sand. Varying ratios of soybean biodiesel (1:2, 1:1, 2:1) were applied in relation to the amount of oil applied and the system was flushed with water at points up to 14 days after initial treatment. The hydrocarbons flushed from the system were then extracted and quantified. In addition, sediment cores were taken from the boxes of sand at the end of the 14 days of treatment and the amount of hydrocarbons at

various levels was quantified to evaluate the degree to which oil, biodiesel or a mixture of the two penetrated the substrate. An interesting finding from the controls was that 25 times more biodiesel was liberated from the biodiesel control than oil from the oil control, with water flushing as the only treatment. This is yet another indication that biodiesel may be removed from the marine environment much more effectively than oil, due to its lower viscosity and higher buoyancy. Additionally, cleaning effectiveness was increased in treatments with a higher biodiesel to oil ratio: the 2:1 treatment removed 7 times more oil than did the 1:2 treatment and 4.5 times more oil than the 1:1 treatment. Removal was greatest in the initial two water flushes regardless of biodiesel-oil ratio.

A larger scale version of the microcosm experiments used a simulated beach comprised of cobbles, gravel, coarse sand and fine sand. Soybean biodiesel was used to clean light crude oil from these substrates, in an effort to evaluate what effect substrates of varying size and density may have on cleaning effectiveness. Results indicated that biodiesel was most effective in cleaning cobbles and fine sand. This efficiency was presumably due to the high surface area to volume ratio of the large cobbles and the tightly packed sand. The other two substrates, gravel and coarse sand, have a lower surface area to volume ratio and do not pack together tightly. On all substrates, the greatest amount of oil was removed with the initial biodiesel application and the water washed from the surface of the substrates had higher concentrations of both oil and biodiesel than did water flushed through the substrates. Subsequent biodiesel applications were decreasingly effective. After seawater was flushed through the substrates, greater quantities of hydrocarbons were recovered from the substrates than from the effluent water. This is significant, as any oil, biodiesel or mixture of the two that absorbs into the sediment cannot be recovered in a real oil spill situation, as it can in an experimental scenario.

In both the microcosm and mesocosm experiments, untreated crude oil resulted in the greatest amount of hydrocarbon penetration in the substrate, suggesting that biodiesel treatments prevented hydrocarbon penetration. The biodiesel controls had a relatively low mobility into the substrates, with most of the biodiesel absorption occurring within the top 5 cm of the substrate. Substrates such as cobbles and fine sand, which had particularly low surface areas in relation to volume, had correspondingly low hydrocarbon penetration. In coarse sand and gravels, which have relatively high surface areas, greater amounts of both biodiesel and biodiesel-

oil mixture were able to penetrate, though this amount was less than resulted from the application of untreated crude oil.

The Pereira and Mudge experiments were an extensive test of biodiesel as an oil spill cleaner. They confirmed that biodiesel is an effective solvent for spilled oil and that greater volumes of biodiesel relative to the amount of oil spilled lead to a greater cleaning effectiveness. Mesocosm experiments offer an experimental baseline for cleaning efficiencies on a variety of substrates with varying pore size, and these results were generally in agreement with the results reported in earlier studies [22]. The authors suggest that based on the performance in laboratory settings, biodiesel should be considered a potential oil spill remediation agent, but warn that results obtained in laboratory settings do not always translate to the field and that biodiesel needs to be trialled on a full-scale oil spill in a marine setting.

#### **2.2.6 Obbard, Ng & Xu, 2004**

Obbard et al. [32] evaluated the efficacy of oil biodegradation by crude palm oil and its fatty acid constituents, myristic, oleic, linoleic and palmitic acid. Light crude oil was applied to moist sand and allowed to weather over 15 days. After the weathering period, the soil was treated with nutrient fertilizers, palm oil or the various fatty acids. Experimental samples were tilled every other day to maintain aerobic conditions and further nutrient supplementation occurred at regular periods throughout the 30 day experiment. Degradation was evaluated by estimating the number of bacteria in a sample, assaying the metabolic activity of the bacteria and chemical analysis of the degraded oil.

Bacterial counts indicated that the crude palm oil enhanced the population of hydrocarbon degrading bacteria, as populations increased 26-fold over the course of 30 days while a control sample (oiled sediment only) increased only 12-fold. However, the addition of fatty acids resulted in bacterial population increases of up to 170-fold, with oleic, palmitic and myristic acids amongst the greatest promoters of bacterial growth. Metabolic analyses showed that at peak metabolic activity, bacteria in the palm oil treated samples were as active as those of the fatty acid treatments and much greater than that of the control treatments. Chemical analysis of the oil after the 30 days indicated that despite the increase in bacterial population and metabolic level, the crude palm oil did not result in a statistically significant increase in biodegradation when compared to the control. However, the fatty acids did

significantly increase biodegradation, as degradation of the relatively susceptible straight alkane components of the oil reached 100%. Biodegradation of the more recalcitrant branched alkanes was again considerably higher in the fatty acid treated samples, but was also significantly greater in the crude palm oil sample than in the control samples.

Both crude palm oil and its constituent fatty acids enhanced bacterial populations and significantly enhanced the metabolic processes of these bacteria when applied to oil-contaminated sands. The fatty acids also resulted in a significantly more complete depletion of all of the tested hydrocarbon molecules. Though crude palm oil did not enhance the depletion of the straight chain alkanes, it did stimulate the loss of the more recalcitrant branched alkanes when compared to controls. The authors suggest that the failure of palm oil samples to become completely depleted of straight alkanes may result from the more complex, heterogeneous carbon source that palm oil represents compared to isolated, simple fatty acids.

Despite this incomplete straight alkane degradation, the authors concluded that both crude palm oil and its constituent fatty acids were of value in oil spill remediation. They also indicated that the less complex fatty acids displayed a synergistic effect to the native hydrocarbon-digesting bacteria by serving as a readily available carbon source and facilitate co-metabolism of the spilled oil. This enhanced biodegradation has been suggested to result from a potential surfactant effect, which increases the dispersion and bioavailability of oil [41, 42].

### **2.2.7 Fernández-Álvarez, Vila, Garrido-Fernandez, Grifoll & Lema, 2006**

In the only study to date that has evaluated biodiesel as an oil spill cleaner in field settings, Fernández-Álvarez et al. [11] examined the effectiveness of sunflower biodiesel on cleaning oil fouled beaches after the spill from the *Prestige* following preliminary manual removal cleaning methods. In addition, the study evaluated other commercial oil spill cleaners and fertilisers, including bacterial inoculations. These products were tested on naturally occurring substrates, including seawater, sand and rocks, as well as experimental granite tiles.

No differences between the various commercial treatments and bacterial inoculations was found by either inshore seawater microbe population estimates or polycyclic

aromatic hydrocarbon (PAH) analysis of pore water collected from the sites. Trials on sand indicated that little oil had penetrated the substrate due to the formation of water-in-oil emulsions, and what oil had penetrated had either been removed in the preliminary cleanup or had already been significantly degraded. Further tests indicated that no evidence of biodegradation enhancement was obvious in samples treated with bioaugmentation products or fertilizers, again, likely due to the already thoroughly weathered nature of the oil. Evaluation of the various products on rocks indicated that the majority of oil removal was due to the physical effects of high pressure application. Again, the extensive weathering of the oil prior to treatment appeared to play a role in retarding both the efficacy of remediation agents and natural biodegradation. On the experimental granite tiles, the cleaning products again performed less effectively than did either the simple nutrient addition or the control tiles in which no treatment was given.

Biodiesel application resulted in much greater success than did the conventional products in this study. The researchers used very low applications rates ( $100 \text{ g m}^{-2}$ ), and observed that the biodiesel rapidly absorbed into the weathered oil that had presented as an asphalt and resin layer hardened on the surface of the rocks. Removal of oil was compared to control rocks which had no cleaning agents applied. Biodiesel-treated rocks decreased in oil coverage from 97% to 16% in 22 months, while the control rocks decreased in coverage from 76% to 26% in 15 months, with no additional loss for the remainder of the 22 month time period. It was estimated that the oil remaining on the biodiesel-treated rocks would be degraded by 26 months, whereas the loss of oil from the control rocks had ceased at 15 months. No such estimate could be formulated for the control rocks, as no change had been observed in oil coverage in the final 6 months of observation.

This study represents the first field application of biodiesel to an actual oil spill, and more importantly, indicates that biodiesel may be effective in enhancing oil biodegradation long after the weathering process has occurred. Even when employed after the utilisation of more traditional methods, biodiesel appears to be effective in reducing the persistence of weathered oil on hard substrates where evaporation often results in a recalcitrant asphalt and resin layer. This finding represents a significant step forward, as few cleaning agents are capable of dealing with weathered oil, especially as early laboratory experiments with biodiesel

suggested that the window of opportunity was narrow [17], demonstrating the value of long term studies and evaluation methods capable of detecting subtle changes.

### **2.2.8 Pasqualino, Montané & Salvadó, 2006**

Pasqualino et al. [20] evaluated the synergistic promotion of biodegradation by the combination of waste cooking oil biodiesel and either petroleum derived gasoline or diesel fuel. Varying ratios of the mineral fuels and the biodiesel were inoculated with bacteria obtained from wastewater sludge and nutrients were added. Biodegradation was determined by measuring CO<sub>2</sub> evolution from the hydrocarbon digesting bacteria, which measures the amount of CO<sub>2</sub>, a waste product of bacterial metabolism, against the amount of carbon in the material being degraded.

Petroleum fuels were found to be miscible in biodiesel in all quantities tested, from 5% to 87.5% (w/w). In trials of both gasoline and diesel, the 100% biodiesel control was biodegraded, as indicated by CO<sub>2</sub> evolution, completely after 28 days and the 100% fuel control was the least degraded (diesel = 50%, gasoline = 56%). The mixtures of fuel and biodiesel degraded according to their biodiesel component; that is, the 87.5% biodiesel in petroleum fuel mixture degraded more completely than any sample except the 100% biodiesel control, and the 5% biodiesel in petroleum fuel sample degraded less than any other sample except the diesel control, over the course of 28 days. These results indicate that biodiesel has a positive synergistic effect on bacterial degradation of petroleum fuels, which means that degradation is not simply enhanced arithmetically. For example, in a 67% biodiesel - 33% gasoline mixture, degradation after 28 days was predicted to be approximately 80% based on the degradation properties of the biodiesel and gasoline. However, in the experiment, the authors report a degradation of approximately 90%, suggesting that biodiesel acts as a synergist, accelerating degradation.

The authors also found that gasoline, a volatile petroleum distillate, experienced enhanced biodegradation when mixed with biodiesel. In spills at sea, volatile distillates are often left to either disperse naturally or evaporate [43]. However, these volatile compounds are often the most toxic [44] and this toxicity may inhibit naturally occurring hydrocarbon-digesting bacteria, reducing the rate of biodegradation rate of any remaining gasoline [45]. Treating such compounds with biodiesel to enhance degradation, rather than allowing the toxic compounds to disperse or evaporate, may be a more ecologically friendly method.

An important recommendation by the authors of this study is that in any spill clean up situation, it is unlikely that all of the spill will be recovered, so regardless of what cleaning method is used, biodiesel application will positively contribute to long term cleaning success through enhanced biodegradation and reducing the toxicity of the most volatile components.

### **2.2.9 Fernández-Álvarez, Vila, Garrido, Grifoll, Feijoo & Lema, 2007**

Fernández-Álvarez et al. [10] followed up their investigation of sunflower biodiesel on oil spilled from the *Prestige* with an evaluation of the effects of biodiesel on artificially oiled tiles located in infralittoral (within the tidal range of the shore) and supra-littoral (above the tidal range of the shore) zones on a beach. Granite tiles to which weathered oil was applied were treated with a variety of bioremediation agents. Supralittoral tiles were treated with biodiesel, a bacterial inoculation, fertiliser or a commercial microbe and nutrient mix and compared to control tiles (only oil, no treatments). Infralittoral tiles (submerged for 12 hours, emerged for 12 hours) were treated with biodiesel, the bacterial inoculation or a nutrient fertiliser and compared to control tiles.

Oil on supralittoral control tiles persisted for almost the entire course of the experiment (360 days). Tiles treated with nutrients and/or microorganisms also remained heavily oiled over the course of the experiment. Biodiesel treated tiles, on the other hand, displayed a significant depletion of oil. However, gravimetric analysis of total petroleum hydrocarbons (TPHs) indicated similar levels among all treatments, indicating that despite the reduced coverage of visual oil on the biodiesel treated tiles, the control tiles had lost a similar amount of oil. The authors explain the differing oil coverage results from the loss of volatile components of the oil coupled with the persistence of asphaltene hydrocarbons on the control tiles, resulting in the tarry appearance despite a loss of some hydrocarbons.

Gravimetric analysis indicated a significant loss of oil in the biodiesel-treated infralittoral tiles compared with the control tiles. Again, nutrient and microorganism treated tiles were not significantly affected.

Tests of the quantity of PAHs in the various treatments found no differences based on treatment or location, but did indicate a nearly complete loss of 16 PAH

compounds. That these losses were independent of location or treatment indicates they were a result of natural processes unaffected by the experimental conditions.

These results demonstrate that of the various treatments, only the biodiesel was effective in promoting removal of the oil from the tiles. It is not surprising that microbial inoculation did not have a significant effect on the degradation rate, as hydrocarbon-digesting bacteria should be prevalent in the area where the experiment was performed due to the oil remaining from the *Prestige* spill. That nutrient addition did not have a significant effect suggests that either the water in the area of the experiment has a nutrient level sufficient to promote bacterial growth or that the native bacteria have adapted to local conditions. A negative finding of the study was that polycyclic aromatic hydrocarbons (PAHs) did not appear to be affected by any of the treatments.

#### **2.2.10 DeMello, Carmichael, Peacock, Nelson, Arey & Reddy, 2007**

DeMello et al. [46] compared the biodegradability and eventual fate of petroleum-derived diesel, an unspecified biodiesel and a diesel-biodiesel blend when they were left in seawater. The diesel-biodiesel blends used were 92% diesel-8% biodiesel (B8) and 75% diesel-25% biodiesel (B25). The degradation of these samples was measured over 53 days in laboratory settings.

Fatty acid methyl esters of the biodiesel were found to degrade in seawater at a rate similar to the *n*-alkane components of the diesel fuel and diesel-biodiesel mixtures. This indicated that they were not playing a role in degradation of either the easy to degrade *n*-alkanes or the more recalcitrant longer carbon chain compounds. However, DeMello et al. found that the neat (100%) biodiesel sample degraded rapidly, losing 90% of its mass in the initial three weeks. Furthermore, the persisting biodiesel is believed to result from adhesion to the surface of the container in which the samples were held and not a result of incomplete degradation. A control biodiesel sample did not degrade, indicating that the losses were caused by the degrading processes of bacteria in the seawater sample.

Biodiesel did not influence the evaporation of diesel components, which is an important characteristic for an oil spill cleaner. If biodiesel were to prevent or reduce the evaporation of volatile components of oil, the toxicity of those volatile compounds could inhibit bacterial growth as well as result in mortality to shoreline biota.

DeMello and colleagues also report that biodiesel may have a stabilising effect on petroleum hydrocarbons, allowing oil droplets to persist as droplets rather than coalesce into a slick. This effect could lead to a greater incorporation of oil into the water column as well as facilitating dispersal by increasing the surface area of the oil. This increased surface area may also be beneficial to the natural degradation process, exposing a greater amount of oil to bacteria. Conversely, increased oil in the water column could promote contamination of the seabed and benthic organisms in nearshore environments.

### **2.2.11 Related studies and the negative environmental effects of vegetable oil**

A number of recent studies have built on work by Obbard et al. [32] by evaluating the efficacy of vegetable oils as cleaners of hydrocarbon pollution [47-49]. These studies all generally support the efficacy of vegetable oils as cleaners, as the vegetable oils show a great degree of solvent capabilities. Also their relatively high viscosity prevents deep penetration into the soil or sand if they land on the seashore. Furthermore, vegetable oils used for hydrocarbon cleaning can be treated to remove PAHs and allow for the reuse of the oil for further cleaning application [50].

Despite these beneficial effects of vegetable oils as cleansers, they may be harmful when applied at sea. Vegetable oil can polymerise in sea water or sediments [51, 52], thus decreasing the oil's susceptibility to biodegradation [53]. Even if spilled vegetable oils do not polymerise, they may be degraded by bacteria, which, combined with the elimination of gas exchange through the vegetable oil slick, rapidly deplete the aquatic environment of oxygen, resulting in anoxic conditions that are deadly to fish and crustaceans [54]. Reports also indicate that because vegetable oil spills have less visual contrast than petroleum oil spills in the marine environment, they often result in greater loss of birds who land in the spill, unaware of its presence [54, 55]. Mudge [55] suggests that in light of the consequences of polymerisation, smothering effects and both direct and non-lethal toxicity of vegetable oils spilled in the marine environment, vegetable oils must not always be considered non-toxic, despite their use as foodstuffs.

This then is a summary of the published research and it is appropriate to turn to some of its limitations.

### **2.3 Limitations of current research**

The use of biodiesel as a cleansing agent for oil spills is still in its infancy, with only 10 studies performed in the past decade. Despite the promising results of these few studies, there is a surprising paucity of published research on the topic of biodiesels as cleansing agents. First, the dearth of fieldwork means that predictions of performance must be extrapolated mainly from laboratory experiments. This is impractical because laboratory experiments, while very useful, are done on a very small scale and often under conditions that can have little bearing on real life applications.

A second major shortcoming is the small range of the available biodiesels that have been evaluated as potential agents in oil spill cleaning. The biodiesels tested to date include soybean, rapeseed/Canola and sunflower, with the most commonly tested being soybean in the United States and rapeseed in Europe [56]. This is a very limited range of the biodiesels produced throughout the world and may overlook beneficial qualities of other biodiesels. For example, rapeseed biodiesel has the greatest viscosity of any commonly produced biodiesel, and both soybean and sunflower biodiesels rank have average viscosity [15]. Additionally, all three of the most commonly tested biodiesels have a high proportion of long chain fatty acids [15]. Biodiesel derived from coconut, in contrast, has the lowest viscosity and a very high proportion of short fatty acid constituents [15], which may allow for greater penetration in coarse sediments and rapid biodegradation.

Other biodiesels may have characteristics particular to the oil they are derived from that make them more or less suitable for application as a bioremediation agent, but these must be tested to find out. Two such biodiesels are those produced from coconut oil and palm oil, as they have very low viscosities [15] and are readily available from Asian countries such as Indonesia and the Philippines. Palm oil has been shown to be comprised of fatty acids that are particularly effective at cleaning oil [32], and coconut oil has a very high proportion of short chain fatty acids that may be amongst the most readily biodegradable [15].

Also limiting the present understanding of biodiesel as an oil spill cleaner are seemingly contradictory findings and data from different studies. For example, Miller & Mudge [17] suggest that there is a narrow temporal window of opportunity for the application of biodiesel after a spill (though they qualify this suggestion by stating that

the window of opportunity is dependent upon the particular conditions), whereas Fernández-Álvarez et al. [10, 11] found biodiesel to be an effective cleaner on heavily weathered oil long after the spill. Similarly, DeMello et al. [46] found that while biodiesel was readily degraded, its degradation did not have any synergistic effect on mineral diesel, though other researchers have found significant co-metabolic effects [20]. These findings may differ based on the abiotic conditions or particular elements of study design, and without further study, these confounding factors can not be identified.

Another effect of the limited number of studies is that each study or group of researchers utilise different experimental methodology and analytical techniques. The loss of oil in response to biodiesel application has been defined photographically [10, 11], gravimetrically [39], chemically [20] and through chromatography [46] and spectrophotometry [9, 17]. This variability in methodology can lead to results that cannot easily be compared between studies.

Further research will clear up these discrepancies, allow for a better standardisation of methodology and facilitate a greater ability to compare results. Also with further research, new findings, such as the recent identification of a surfactant behaviour of biodiesel [46], will come to light and be evaluated, thus broadening the understanding of the abilities and limitations of applying biodiesel as an oil spill bioremediation agent.

## **2.4 Recommendations**

Considering the data that have been presented, we recommend the following to further develop the understanding and practical application of the use of biodiesel as an oil spill cleaning agent:

1. Evaluate different biodiesels, particularly palm oil and coconut biodiesels;
2. Evaluate the effectiveness of biodiesels on a range of oils that may potentially be spilled;
3. Follow up laboratory experiments with field experiments;
4. Develop standardised set of methods to make results more understandable, communicable and comparable to that of other researchers;
5. Elucidate how biodiesel treated spills may react in climates ranging from tropical to subtropical to temperate;
6. Expand the scope of toxicity testing to include organisms likely to be affected by an oil spill on an Australian or similar Pacific region shoreline;
7. Develop an understanding of biodiesel's "window of opportunity" for application after the spill in oil spill response;
8. Develop a response protocol and evaluate it in a field setting;
9. Further understand the role biodegradation during storage of biodiesels may play in the suitability of biodiesel as an oil spill response agent;
10. Determine in more detail the environmental benefit and detriment of biodiesel application in oil spill response.

From the researchers' point of view, these recommendations are all practicable and balanced.

## **3 Report on Empirical Laboratory Research**

### **3.1 Introduction**

Biodiesels have potentially important roles in coastal remediation involving the clean up of oil spills, both with regard to their solvent behaviour [8, 22] and their relatively environmentally benign chemical properties [28] (§ 2.2.1 through 2.2.11). In contrast to conventional oil spill remediation techniques, which may result in more damage to the marine environment than that caused by the oil by itself, biodiesel has been shown to work in two ways to facilitate the removal of oil without causing further environmental damage. The first function of biodiesel is to dissolve and mobilise spilled oil [10]. This prevents the oil from doing environmental harm, including being stranded on the shore, where it can form a resinous asphalt layer on rock and sand surfaces [10]. The second function of biodiesel is to serve as a readily degraded carbon source, which facilitates co-metabolism of the hydrocarbons in the spilled oil, allowing for hydrocarbon digesting bacteria to break down the mixture of spilled oil and biodiesel much more quickly than untreated oil [22].

While biodiesels offer promise, empirical studies to determine their effectiveness (§ 2.2), relative toxicity (§ 2.1.2.4) price and availability (Appendix B, §5.2) are scarce. Furthermore, experimental approaches for assessing their effectiveness are currently developmental and vary markedly amongst researchers. The derivation of accurate, relevant and credible methodologies that are easily reproducible is important in quantification of remediation effectiveness.

One of the systematic methodological difficulties in evaluating biodiesel as an oil spill cleaner is that of defining “effectiveness.” An example is a study in Japan that gauged the “effectiveness” of a bacterial method of oil removal by monitoring the change in colour of rocks stained with oil in response to treatment [57] and a control. The bacterial treatment was suggested to have cleaned the rocks, but no information was sought about the fate of the pollutant or the impact on the marine environment. Thus while the treatment

was effective within the framework of the experiment the assessment was not effective in determining the overall efficacy of the treatment. Other studies have quantified the amount of hydrocarbons in water that has been flushed through oiled substrates [17], though this is a measure that can not be done in situ on an oil affected beach.

These were problems identified in the Literature Review, above, and they were also problems in setting up the laboratory system for these particular biodiesel experiments. In the present empirical study, we evaluate in the laboratory the effectiveness of two biodiesels, which, to our knowledge, have not been tested previously for effectiveness in removing oil from several hard and soft substrates. No evaluation of the impacts of either the biodiesel itself or the biodiesel-treated oil on marine communities is attempted in these present experiments.

The present study provides a set of replicated experiments concerning the ability of various cleaning agents to remove bunker oil, determined by percent oil removed, from a range of environmentally relevant substrates under controlled laboratory conditions. The laboratory conditions were established to mimic a marine oil spill scenario while removing the inherent variability and stochasticity of such events. Thus, while the results of this experiment may offer some insight on how biodiesel facilitates the removal of spilled oil, they may not be entirely applicable to variations in conditions that have been neither controlled nor accounted for.

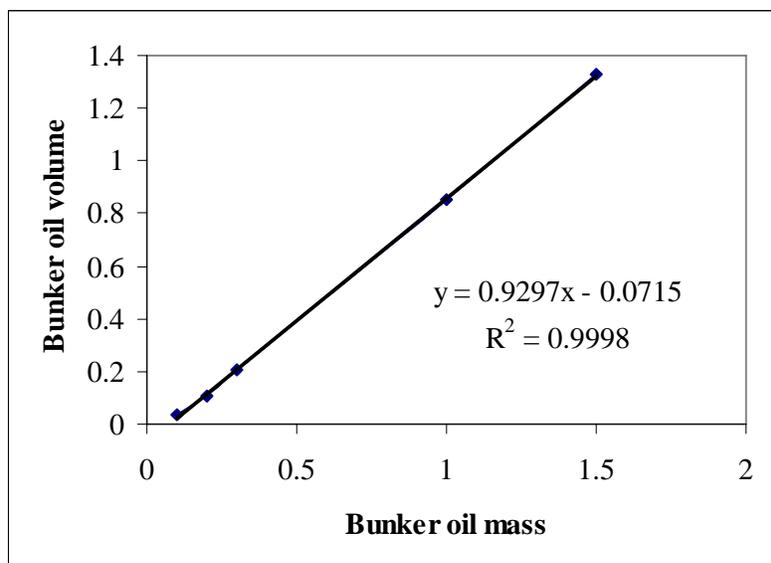
The basic experiment used eight substrates and seven cleaners of which two were the biodiesels (i.e., palm oil and coconut oil). The details of the substrates and cleaners are set out in Table 1. The effectiveness of the cleaners was tested on substrates contaminated with bunker oil – type 380 centi stokes (cst).

## **3.2 Materials and Methods**

### **3.2.1 Laboratory protocol**

Experimental conditions assumed an intertidal scenario in which substrates would be damp following either wave splash or from the retreating tide. Therefore all substrates were moistened using seawater prior to analysis. All experiments were conducted in a temperature controlled room at 20°C to mimic average east coast maritime conditions. Experiments were conducted at the University of Queensland Moreton Bay Research Station to facilitate access to marine substrates and fresh seawater. A weathering period for the bunker oil of 48 hours was used to simulate field response times expected. Weathering was achieved by decanting a volume of bunker oil into a beaker and leaving it uncovered in a fume cupboard for the allotted time.

Weathered bunker oil was applied to selected substrates in the following manner: pre-weighed ( $\pm 0.001$  g) hard substrates, sized to fit within a 50 ml Falcon tube, were treated by a “dip and drip” method in which forceps were used to entirely immerse the substrates in a beaker of oil (Figure 1), thus maximising substrate contact with oil. The oiled substrate was then suspended by forceps over a tray and allowed to drip. Once no drips occurred for 30 secs, each treated sample was then weighed in a tared (weight zeroed) 50 ml Falcon tube prior to cleaning agent application.



**Figure 2. Calibration curve for bunker oil volume vs bunker oil mass to enable calculation of application rate of cleaning agents. Correlation coefficient and linear relationship are shown on the plot.**

The treatment of soft substrates (mud and sand) differed in that they could not be dipped into the beaker of oil. Rather, a 20 ml syringe was used to deliver known volumes of bunker oil to pre-weighed soft substrates.

Cleaning agents were then applied to all substrates using a volumetric dispenser. Application rates of the commercial cleaners and biodiesels were not constant but calculated on a volume/volume ratio based on directions of manufacturers (CytoSol, CoreExit 9500, Biosolve, CT-18, Palmolive) or on rates used in previous publications (biodiesels) to ensure an effective comparison. Oil volume was estimated using the known weight of oil obtained by subtraction set against a calibration curve for weight vs. volume previously established (Figure 2). The application of cleaner was followed by seawater application via a volumetric dispenser approximately 5 mins after application, again to mimic field applications techniques on a small scale.

**Table 1.** Cleaners and substrates used in experiments to test the relative efficacy of biodiesel as an agent for use in oil spill cleanup.

Substrate/ Cleaner	Approved type	Obtained from/Manufactured by
Rock	Granite	All Stone Creations, Hendra, QLD
Concrete	Marine grade concrete	One Mile Jetty, North Stradbroke Island, QLD
Wood	<i>Avicennia</i> pneumatophores	Baffle Creek, near Bundaberg, QLD
Aluminium	Aluminium boat (weathered)	Bay Scrap Metal & Recyclers, Redcliffe, QLD
Fibreglass	Fibreglass (marine grade)	Cunningham's Marine, Redcliffe, QLD
Coral	Aged coral rubble ( <i>Acropora</i> spp.)	One Mile Beach, North Stradbroke Island, QLD
Sand	Silica beach sand	Polka Point, North Stradbroke Island, QLD
Silt	Mangrove mud	Polka Point, North Stradbroke Island, QLD
Degreaser 1	Detergent (Palmolive)	Colgate-Palmolive Australia, Sydney, NSW
Degreaser 2	CT18	Applied Australia LTD, Clayton South, VIC
Surface Cleaner	Biosolve	Pacific Biosolve, Cronulla, NSW
Dispersant	Corexit 9500	Nalco Energy Services, Sugar Land, TX, USA
Shore Cleaner	CytoSol	CytoCulture Environmental Biotechnology, Richmond, CA, USA
Biodiesel 1	Palm oil based	Natural Fuels LTD, Darwin, NT
Biodiesel 2	Coconut oil based	Chemrez Inc, Manila, Philippines

Following the seawater rinsing, the fluid mixture of seawater and cleaning agent/mobilised oil mixture was then decanted, substrate samples reweighed ( $\pm 0.001$  g). Total hydrocarbon extraction analysis was then performed on all supernatants using hexane extraction followed by drying of 1 ml samples of the settled hexane-oil fraction in 1 ml Eppendorf tubes, and reweighing to determine the mass yield of oil [19] and contaminants (Figure 3, 4).

### 3.2.2 Quality control and Data analysis

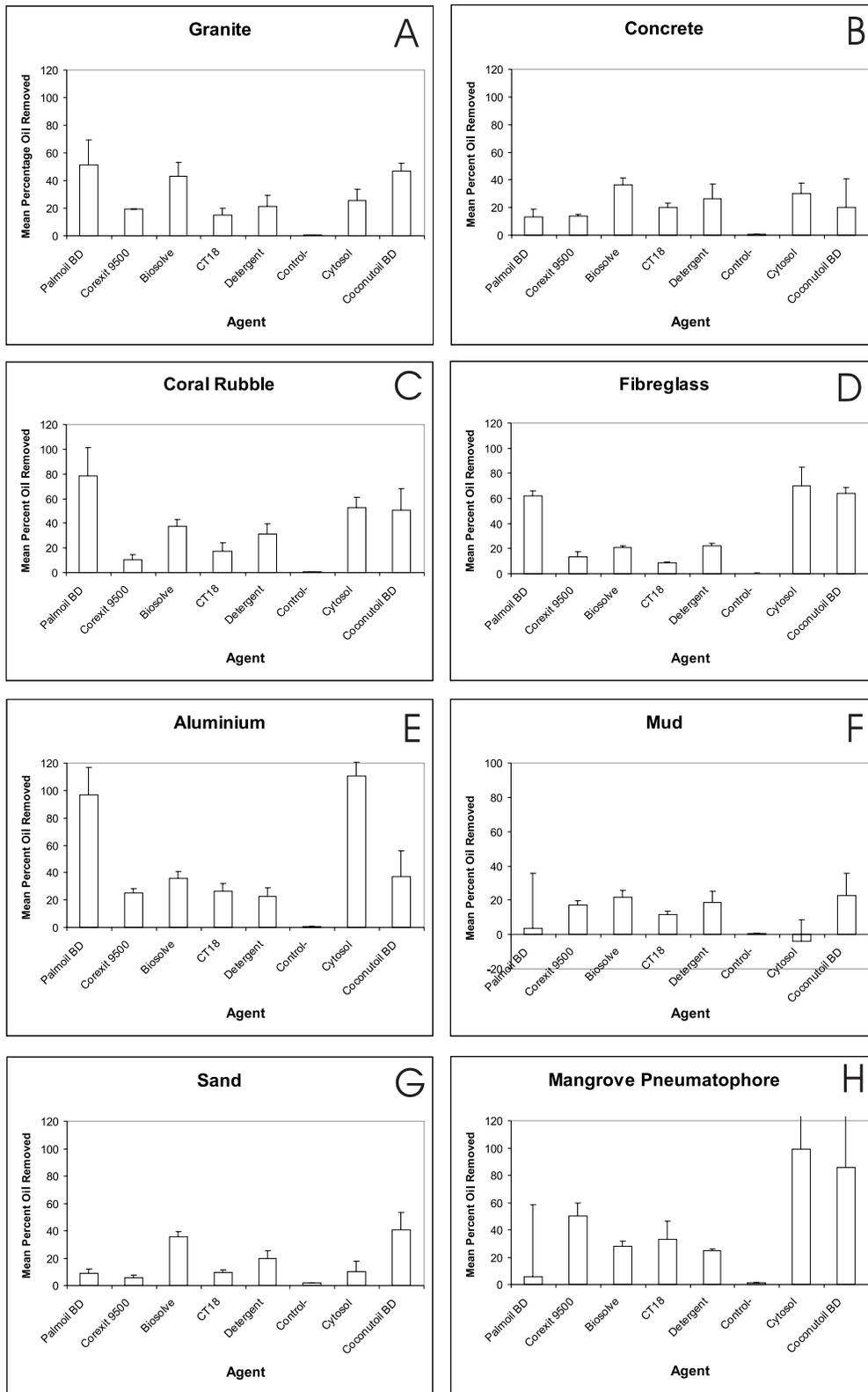
As temperature influences the viscosity of bunker oil and biodiesel all experiments were conducted in a constant temperature room at 20°C. To enable statistical comparisons among substrates and cleaning agents, three replicates of each treatment were used. Biodiesel only (biodiesel agent sprayed on to a substrate not coated in bunker oil (C+) and seawater only controls (C-)) were included to determine the effect of the biodiesel on hydrocarbon extraction and effects of physical removal of oil by the spray application method. Control values were used to correct the mass values for dried substrates following hexane extraction. The seawater only controls were plotted for comparison with the various cleaning agents.

Statistical analysis involved factorial ANOVAs (a statistical procedure for judging the differences among mean values for a range of treatments) and differences were accepted as significant at the  $p < 0.05$  level. Variances were checked for homogeneity and normality.

### **3.3 Results**

#### **3.3.1 Substrate performance**

Oil was recovered from all substrates in excess of that amount recovered in seawater controls (Figure 3). Highest relative recovery occurred from solid substrates whereas recovery from sediments was low (and in one case for mud, negative). It appears that some cleaners performed well against one oiled substrate while performing less well against another oiled substrate. For example palm oil biodiesel performed well against oil on granite (Fig 3A) but less well on oil on concrete (Fig 3B). Despite the strenuous efforts made by the investigation team differences in performance among replicates resulted in high variance (see the vertical error bars on the plots) which precluded the application of robust parametric statistical tests. The results are set out in Figure 3.



**Figure 3A-H.** Plots of mean percentage of oil removed against the cleaning agent used for each substrate. Error bars = standard error.

### **3.3.2 Cleaning agent performance**

#### ***3.3.2.1 Palm oil biodiesel***

Palm oil biodiesel performed best on aluminium removing an average of 98% of bunker oil (Fig. 3E). It was also the most effective agent on granite (Fig. 3A). It ranked second in terms of the agents used in removal of oil from coral rubble (Fig. 3C), and ranked third on fibreglass (Fig. 3D) behind other biodiesel based products. It performed relatively poorly on soft sediment treatments (Fig. 3F, G) and mangrove pneumatophores (Fig. 3H) (more biologically active substrates), and also ranked in the middle of the field for concrete (Fig. 3B).

#### ***3.3.2.2 Cytosol***

Cytosol was the most effective agent in the removal of oil from aluminium (Fig. 3E), fibreglass (Fig. 3D), coral rubble (Fig. 3C), concrete (Fig. 3B) and mangrove pneumatophores (Fig. 3H). It also performed well on granite (Fig. 3A), but less well on sand (Fig. 3G) and mud (Fig 3F).

#### ***3.3.2.3 Coconut oil biodiesel***

Coconut oil biodiesel cleaned most effectively on hard substrates, particularly mangrove pneumatophores (Fig. 5H), aluminium (Fig. 5E), fibreglass (Fig. 5D) and coral rubble (Fig. 5C). It ranked in the mid range of effectiveness against oil present on other substrates. Like other biodiesel based products, it performed poorly on soft substrates.

#### ***3.3.2.4 Biosolve***

Biosolve performed in the middle range of effectiveness on most substrates. It was the best performer for cleaning sand (Fig. 3G), while it did creditably against coral rubble (Fig. 3C).

### **3.3.2.5 Corexit 9500**

Corexit 9500 was very effective at removing oil from most substrates, even when applied at the very low rates recommended. Its effectiveness is not accurately reflected in the accompanying graphs because it has a surfactant component that drives all the oil into the water fraction of the treated solution leaving almost none in the hexane fraction and thus available to be recorded following drying. This performance only became apparent after Falcon tubes were left overnight, after which the surfactant molecules appeared to lose their hold, allowing the oil that had been dispersed into the water to be taken up into the hexane fraction. The relevance of this is that samples of extracted oil were taken from the hexane fraction at a set period after the test, meaning that when the samples were removed little oil or biodiesel was present in them because it was temporarily dispersed in the water fraction.

### **3.3.2.6 CT18 and Palmolive**

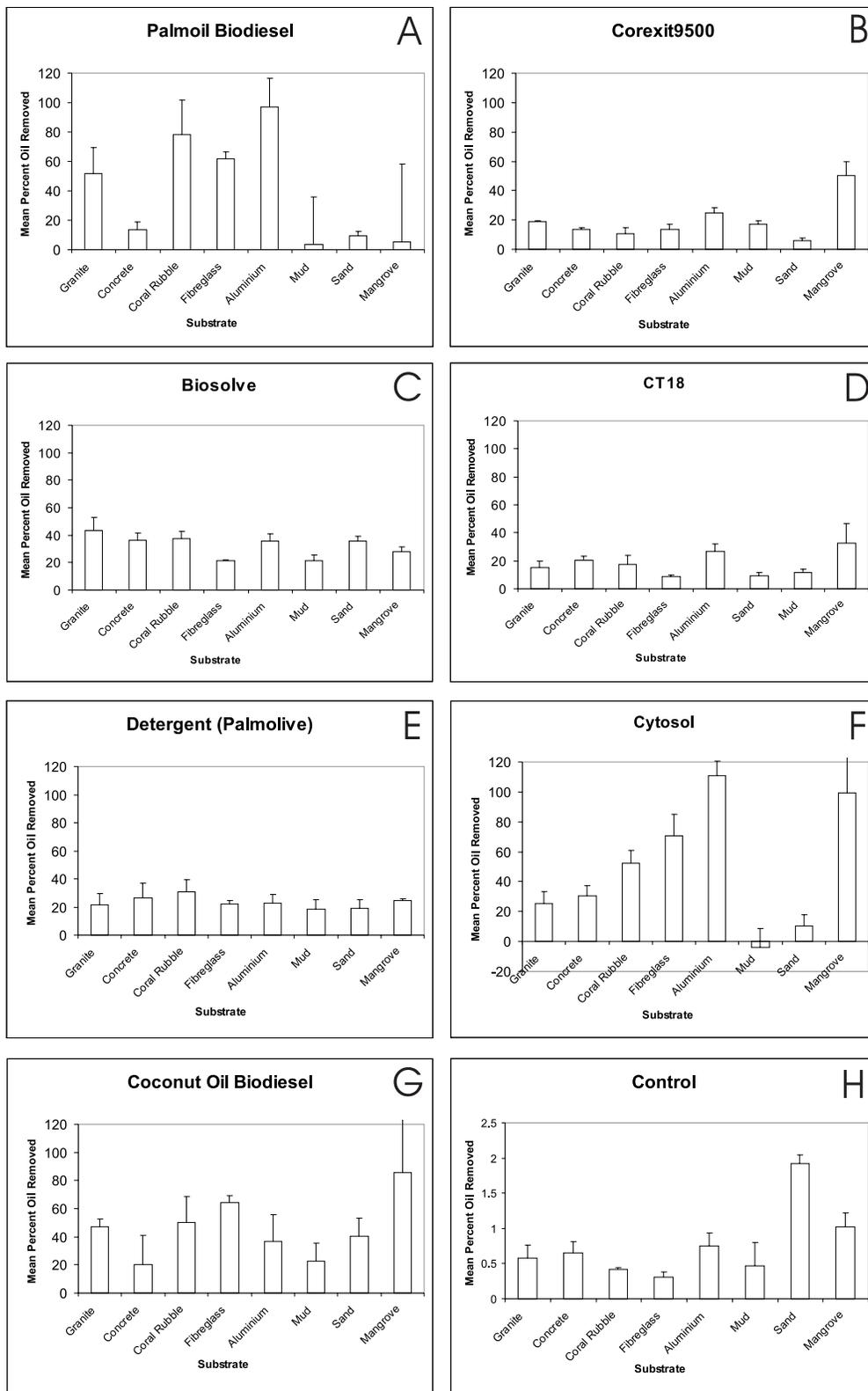
The commercially available detergent/degreasers performed less well than the solvent-based agents. Only in the case of mud, sand and mangrove pneumatophores did their cleaning properties meet or exceed those of any of the solvent-based agents.

### **3.3.2.7 Seawater Control**

Seawater control alone resulted in the removal of only a small percentage of oil from substrates. The use of seawater appeared to be most effective against oil on sand, however the recovery rate was so low (ca 2%) that the energy costs of pumping sufficient water are questionable.

## **3.3.3 Performance of Cleaning Agents Among substrates**

It is clear that some substrates are more easily cleaned than others (see Section 3.3.5) for comment on these. Figure 4 A-H shows the responses to the various cleaning agents of each substrate used. These are the same data presented in Figure 3A-H, but in a different arrangement to facilitate the comparison.

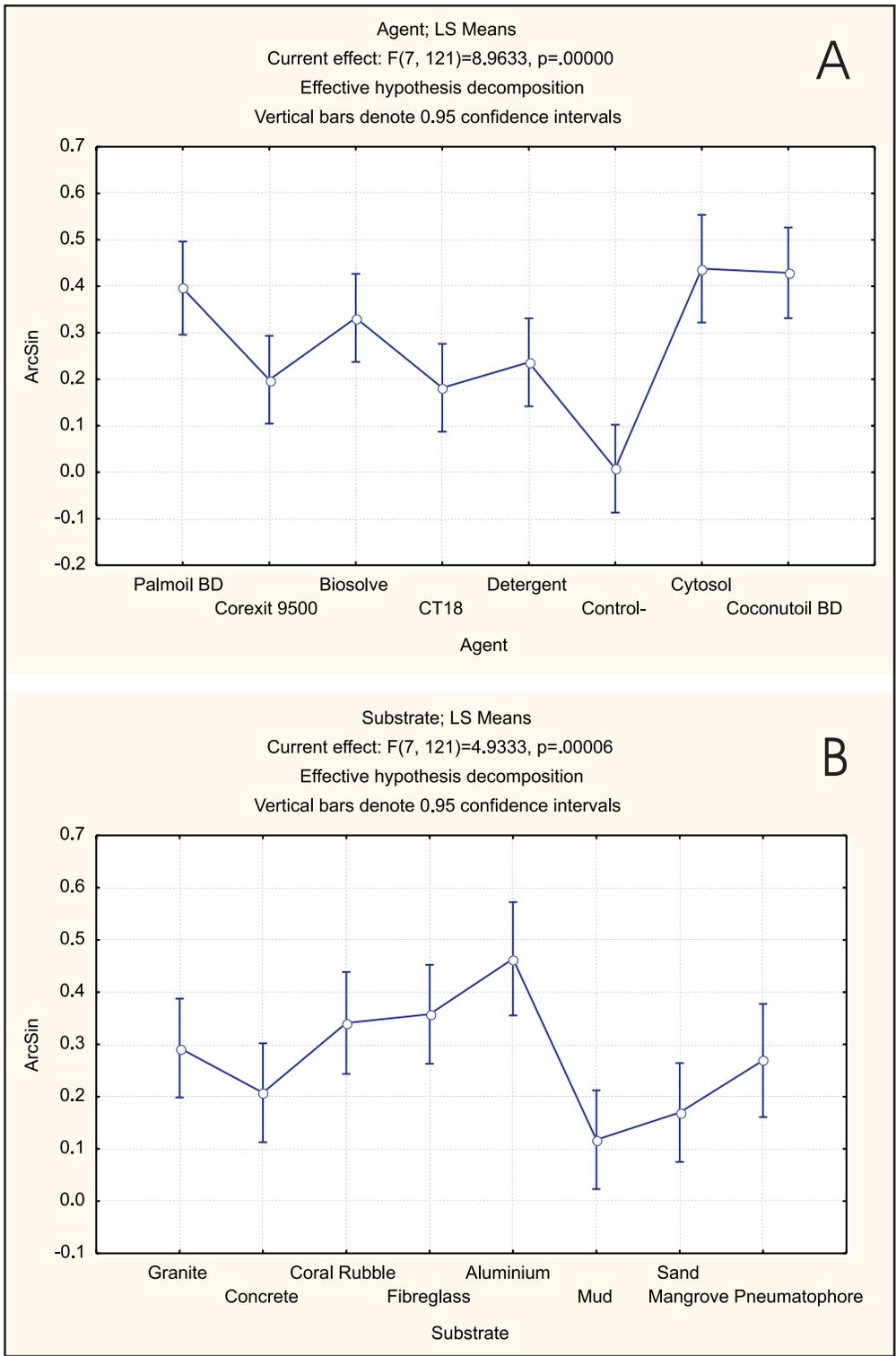


**Figure 4.** Plots of mean percentage of oil removed against different substrates for each cleaning agent used. Error bars = standard error.

### **3.3.4 Statistical comparisons among agents and substrates**

The following graphs (Figure 5A,B) are the result of a two way analysis of variance in which the factors Substrate and Agent yielded a significant ( $p < 0.00001$ ) interaction factor in explaining the dependent factor arcsine transformed values of proportion of oil removed ( $p < 0.00001$ ). This indicates that the relationship is complex, being case dependent, i.e. a particular agent works well on one substrate but not as well on another, but for another agent the result is significantly different. The complete output of the analysis is very complex. Moreover with the high level of variability in the results (large SE values) and the low level of replication ( $n = 3$ ) it is not unreasonable at present to defer attempting to interpret interaction factors. Rather we have given the results for each factor as though it and it alone was significant. This allows us to describe the general trend in cleaning agent effectiveness and the general trend in the ease with which various substrates were cleaned. These data can facilitate the planning of further experiments in which the number of agents and substrates used can be reduced in favour of higher replication.

Biodiesel products (Coconut Oil BD, Cytosol and Palm oil BD) exceeded the performance of degreasers and detergent (Fig 7A). They also appeared to outperform Corexit 9500, however this was due to an experimental anomaly due to the way in which Corexit 9500 works (see below) and the method by which cleaning effectiveness was measured. The control values indicate that seawater alone removes very little oil from the substrates. Of all the substrates tested oil was removed most easily from aluminium (Figure 5B). On the other hand, soils and pneumatophores (mangrove roots) were the least effectively cleaned substrates.



**Figure 5. A.** Plot of relative effectiveness of cleaning agents in removing bunker oil indicating that biodiesel products ranked top in their ability to remove oil from the various substrates. It is important to note that Corexit 9500 appears to perform poorly because its surfactant drives the oil and biodiesel into the water fraction in hexane

*separations. After 24hrs the oil in the water fraction moves into the hexane fraction. B. Plot of relative effectiveness with which the agents used clean the substrates, indicating that mud and sand were least cleaned by cleaning agents and aluminium was most effectively cleaned by the agents. Y axis values are arcsine transformed proportions of oil removed.*

### **3.3.5 General Observations**

The hexane fraction of solutions involving biodiesel had a characteristic bubbly appearance. This was observed in all biodiesel treatments and seems to be an interaction between the bunker oil, biodiesel and hexane. The implications of this observation are not clear, but prompted our interest in the viscosity of any oil/cleaning agent mixture liberated through the cleaning process. The propensity of oil-cleaning agent mixtures to combine with other materials is also of considerable interest.

Adherence of oil to the sides of Falcon tubes proved problematic, particularly when very small volumes of cleaning agent were applied. Even when agitated these small volumes were unlikely to reach such adherent oil. Variability through this effect will have had a direct effect on the amount of oil available to be recovered by the agent.

### **3.3.6 Caveats**

Attempts to provide statistically robust tests using three replicates were hampered by heterogeneity of the substrates used in the experiments. It proved difficult to achieve completely uniform substrate sizes and shapes, despite the best efforts of the team. Variation in substrates likely explains the majority of the variation in results, which led to high values of standard error in many cases. Indeed, in a few instances extracted weight exceeded the weight of oil present on the substrate. This led to some error estimates producing a mean that exceeded 100% removal, indicating that more had been removed than was originally present, meaning in turn that additional material had been

removed. This clearly was a distortion and a likely cause of the major variation.

### **3.4 Discussion**

#### **3.4.1 Relative effectiveness of biodiesels as cleaning agents**

The experimental evidence indicates that biodiesels and biodiesel derived cleaning agents provide comparable or better clean up rates for most substrates compared with most currently used agents, including detergents and degreasers. They are likely to be less biologically active and so are worthy of further investigation in scaled up field trials to gauge their operational efficacy and their effect on the marine environment.

It is important to note that Corexit 9500 appeared to us remarkably effective in removing oil from most substrates tested here. However its action of driving the oil into the water fraction by virtue of its surfactant moieties means that during treatment, particularly of soft sediments, the oil may be driven deep into sediments, where it is less available for physical recovery or remediation by aerobic bacterial processes. Hence while Corexit9500 is effective as an agent for cleaning hard substrates its employment must be considered in balance with both its potential toxicity and its tendency to drive pollutants to deeper less accessible situations.

##### **3.4.1.1 Recommended Research**

Within the time and budgetary limitations of the project, it was necessary to reach a compromise between testing an environmentally realistic range of cleaning agents and substrates and achieving effective replication. Substrate heterogeneity and adherence of some oil to the Falcon tubes rendered the data too high in variance for comprehensive statistical comparison. A greater level of replication, on more homogeneous substrates is required to provide a definitive test of relative effectiveness of cleaning agents. Furthermore cost benefit analysis based on quantity of oil removed per dollar will provide a more effective index for use by agencies involved with oil spill cleanup. Such agencies also need to be able to ensure swift and secure supply, based on either stockpiles or guaranteed supply. Both need to be assessed in determining the efficacy of the various cleaning agents for use in oil spills.

## **3.5 Conclusion & Recommendations**

### **3.5.1 Conclusions**

1. Biodiesel cleaning agents outperformed most other agents presently used in the clean up of oil spills;
2. Different substrates respond to agents differently in terms of the facility with which oil may be removed from them;
3. Substrate heterogeneity possibly contributed to the scale of variance in the results;
4. Standardisation of experimental methods prevents judgements being made on how application methods might impact treatment or results.

### **3.5.2 Recommendations**

1. Field tests of biodiesel based cleaning agents need to be conducted to scale up their performance;
2. Toxicity/ morbidity tests of biodiesel agents need to be undertaken as a matter of priority, and should investigate smothering as well as toxicological properties;
3. Special consideration needs to be given to the preferred outcomes in the clean up of soft sediments and porous substrates;
4. Increase the number of replicates to enhance the statistical power of future experiments.

## **4 Overall Conclusions and Recommendations**

### ***4.1 Conclusions***

In light of combined findings of the literature review and the laboratory experiment, we conclude that biodiesel is an effective cleaner for oil spills in many situations. Both palm biodiesel and coconut biodiesel, as well as the biodiesel-based proprietary formulation Cytosol, performed very well in the experimental tests. This effectiveness was primarily due to the solvent and viscosity reducing behaviour of the biodiesels, as their application onto the oiled substrates resulted in a mixture that was considerably more mobile and fluid than the oil alone. Palm oil biodiesel performed very well on granite, coral rubble, fibreglass and aluminium, and relatively poorly on concrete, sand, mud and mangrove pneumatophores. On the other hand, whereas coconut biodiesel performed very well on most of the substrates, save aluminium, palm oil biodiesel outperformed coconut oil on granite and coral rubble. However, the biodiesels were outperformed by other cleaning agents on some substrates such as fibreglass. This all suggests that biodiesel may be an effective choice on certain substrates and less effective on others.

In addition to the effectiveness of biodiesel as a cleaner, as evidenced by our experiment, the extant literature on biodiesel suggests that several properties of biodiesel are particularly favourable for this application. First, the reduction in viscosity imparted by the biodiesel may ameliorate the smothering effect of spilled oil on intertidal organisms. Secondly, the toxicology trials that have been performed indicate that biodiesel has a very low toxicity. Also, biodiesel has been reported to exhibit a co-metabolic synergistic effect which was not in the scope of our experiment, but may prove invaluable to oil spill response. These factors may work in concert, as biodiesel application may help to reduce the toxic effects of oil simply through dispersal and dilution, while also expediting degradation of the oil, thus reducing the amount of time it affects the marine environment.

Biodiesel has a variety of beneficial properties that warrant further research into its use as an oil spill remediation agent. The application of biodiesel in the event of a spill is still currently hampered by a lack of understanding of the effects of biodiesel application on a large scale and over the long term.

#### ***4.2 Recommendations***

One of the strongest recommendations that can be made regarding subsequent research into the efficacy of biodiesel as an oil spill cleaning agent is that studies need to move out of laboratory into the field. Laboratory studies in controlled conditions limit confounding factors but also fail to indicate how real world performance might be affected by such confounding conditions. Of key concern to developing a practical field work trial are the following:

- Determine realistic application rates;
- Develop, optimise and evaluate field work protocol;
- Develop field work protocol into oil spill response protocol.

Several key systematic concerns must be addressed in future research as well. Of critical importance is a standardisation of methodology and analytical techniques. Without standardisation, comparing results from one study to another will be difficult and potentially important findings may be obscured. Additionally, experiments need to be expanded to large scale and long term studies with high levels of replication in order to ensure statistically robust tests. Experiments run on a large scale and with ample replication prevent either small systematic errors or anomalous data from corrupting results and obfuscating comparisons. Addressing these concerns may help to resolve contradictory findings from previous studies, such as the differences reported in the “window of opportunity” of biodiesel application, an indication that biodiesel application must occur soon after an oil spill in order to be effective.

Expanding the scope of research also allows for evaluating a wider variety of conditions. An oil spill into the Australian marine environment may potentially affect animals in tropical, sub-tropical or temperate waters. No studies have

examined how biodiesels perform at different temperatures either as agents of oil removal or potential toxicants for marine organisms.

Practical considerations such as the rapid mobilisation of large quantities of biodiesel in the event of an oil spill versus the storage of large quantities must be evaluated. Biodiesel has a limited shelf-life being prone to degradation. Whether biodiesel that is unsuitable for fuel use because of its limited shelf life may be used for cleaning spilled oil is an important consideration. As the shelf life of biodiesel is based on its high degree of biodegradability, partially degraded biodiesel may be more effective at cleaning oil spills as it may play host to a population of hydrocarbon-degrading bacteria before it is applied. Conversely, partial degradation (shelf life) may have a negative effect on the ability for biodiesel to act as a solvent; but this would require investigation.

Other practical concerns that must be addressed include the treatment or disposal of wastes after the application of biodiesel to an oil affected area. Net environmental benefit analysis must be performed to determine if biodiesel treated oil can be allowed to decompose and disperse naturally or if removal of fouled substrates (where possible) is the more desirable course of action.

## **APPENDIX**

## **5 Appendix A: Background Summary Concerning Oil Spills and Oil Spill Responses**

### ***5.1 Effects of oil spills in the marine environment***

Oil spills in marine habitats have catastrophic effects on the animals, the substrate and the overall ecology of the environment. Smothering [58] and toxicity [17, 59] present the greatest hazards to shoreline biota, and the persistence of heavy oils can prolong the threat [59, 60]. Petroleum products exhibit significant toxicity to aquatic organisms, ranging from severe acute toxicity in the more volatile short-chain hydrocarbons [27, 61] to chronic toxicity resulting from prolonged exposure to long-chain hydrocarbons that persist on top of or absorbed into the sediment [59, 62, 63]. Penetration of oil into the substrate can promote the persistence of oil as well as increase the difficulty of removal and reduce the efficiency of the biodegradation process [17]. Furthermore, a process known as chronic re-oiling, which is the subsequent release of oil that is absorbed into the sediment over the course of months to years, can further stress the environment for extended periods of time [63-65].

Oil spills have also been shown to have significant impact on the ecology of marine environments. In addition to resulting in the mass mortality or morbidity of particularly sensitive organisms, oil spills may allow other, less sensitive organisms to thrive due to the removal of competition, resulting in a persistent phase shift of the system [66-68]. While the eradication of a particular species of algae or amphipod does not garner the same attention as oiled birds and beaches, the disruption of the interactions of organisms in a particular system can have dramatic consequences. For example, the *Tsesis* oil spill in 1977 contributed to an elevated level of fungal infection on herring eggs, due to the massive die off of an amphipod that feeds on fungus [69]. Research investigating the effects of spilled oil on corals indicate that the energy invested into recovery from oiling events reduces energy available for vital functions such as skeletal growth and reproduction [64, 70]. Such complex interactions are common in marine systems, and they are often not well understood until a major disturbance occurs.

## 5.2 Oil spill trends

Despite great increases in worldwide movement of oil, both the occurrence and volume of oil spilled into the marine environment have decreased greatly over the past 40 years [71, 72]. The vast majority of spills are small (<7 tonnes), though medium (7-700 tonnes) and large spills (>700 tonnes) contribute greater volumes and still occur (Fig. 1). From 2000-2004, a worldwide average of 18.4 oil spills greater than 7 tonnes (medium-large) occurred per year [72]. Oil spilled from tankers accounts for the majority of oil spilled into the marine environment worldwide [71], and as a result, even a small number of incidents can result in large quantities of oil spilled. For example, the 63,000 tonnes of oil spilled from the *Prestige*, one of 15 medium to large spills to occur in 2002, accounted for approximately 94% of the oil spilled worldwide that year (Fig. 2) [72]. Tankers carrying crude oil present a particular danger, as crude contains a large amount of recalcitrant hydrocarbon compounds, which are absent in the more refined petroleum products. Other significant sources of oil spilled into the ocean include illegal dumping from vessels, pipeline ruptures, oil well failures, accidents during bunkering and sunken ships that still contain oil [71, 73].

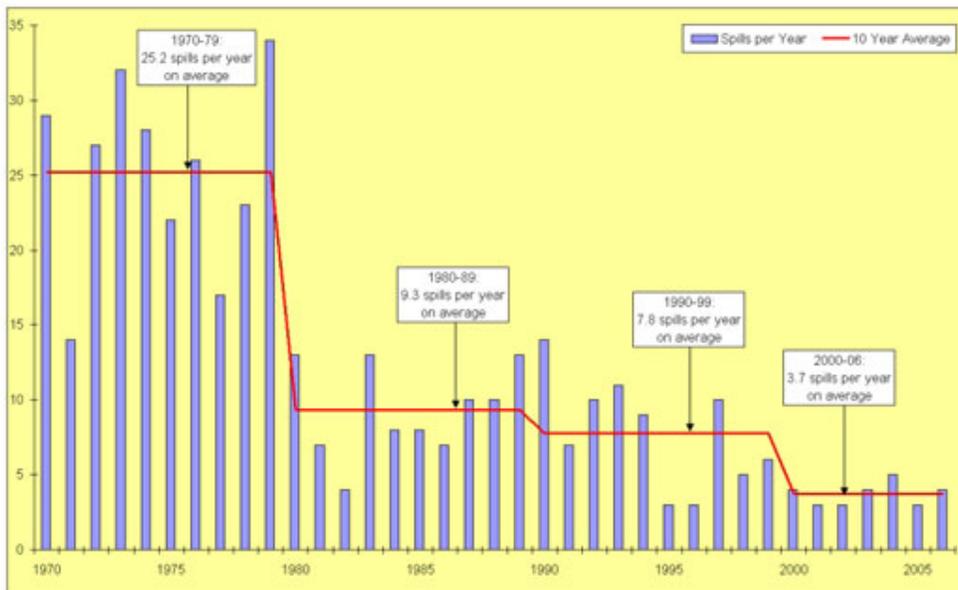
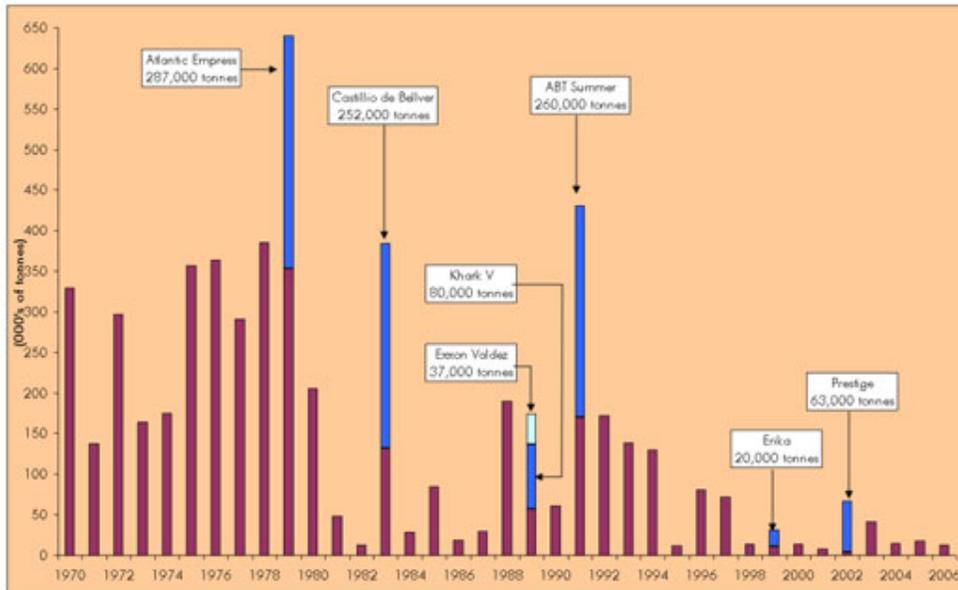


Figure 1. Occurrence of spills per year, with 10 year trend lines. From ITOPF 2007 statistics [74].



**Figure 2. The amount of oil in thousands of tonnes spilled per year. From ITOPF 2007 statistics [74].**

In recent years, oil spills have occurred throughout the world, with the United States, South Korea, Brazil and coastal European countries suffering the bulk of marine oil spills [72, 74, 75]. Australia has been relatively free of major oil spills, with the *Sanko Harvest* and *Kirki*, both in Western Australia, representing the only spills of greater than 700 tonnes in the past 20 years. In that time period, at least 12 other oil spills from ships have occurred in Australia, ranging from 2-600 tonnes [76]. However, this relatively low oil spill frequency does not mitigate the seriousness of the threat oil spills represent to the marine environment, as illustrated by the June 2007 *Pasha Bulker* grounding [76]. The Australian coast is rich in ecologically sensitive habitats such as mangrove stands and coral reefs, as well as beaches that are culturally important and economically significant tourism destinations. Of even greater concern is the vulnerability of the Great Barrier Reef one of the premier eco-tourism destinations in the world and home to the largest assemblage of living organisms anywhere. The risk of oil spills on the Great Barrier Reef is of particular concern, as it lies within the major shipping lane between Australia and Asia, with many ships travelling between the reef and the mainland (Fig. 2) [77, 78].



Figure 2. Map of shipping routes around the Great Barrier Reef [78].

### 5.3 Common methods of oil spill remediation

Those responding to an oil spill must consider a wide variety of factors, such as the local and national response plans, the location of the spill, the weather conditions, the type of oil spilled, manpower and machinery availability, and other variables [17, 74]. Each oil spill needs to be addressed in a manner specific to its particular conditions, and as a result, a range of oil spill responses have been developed. Agencies involved in oil spill response are often pressured by the negative publicity

from the media, public insistence to “do something” and a desire to make decisions that appear proactive and aggressive in the clean up effort [79, 80]. This can lead to poorly planned response decisions that result in the adoption of clean up methods that cause greater ecological damage than the oil spill itself [81-83]. The risk of exacerbating environmental damage makes the consideration of the benefits and limitations of oil spill response essential and the utilisation of methods appropriate to the individual situation.

The AMSA National Plan advises a range of strategies in dealing with an oil spill [Section 3, Page 4 of 7, *National Marine Oil Spill Contingency Plan*, AUSTRALIA Version 2.0]. It is stated that “all may be effective to a degree according to the conditions prevailing and the sensitivity of the environment under threat”. The response options include:

- surveillance;
- control and recovery;
- application of dispersant;
- in-situ burning;
- shoreline cleanup; and
- bioremediation.

They advise that response managers should consider the degree of risk associated with cleanup operations as a function of the:

- type of oil spilled;
- size of the spill;
- location of the spill;
- circumstances of the spill; and
- weather conditions.

### **5.3.1 Chemical dispersants**

The application of chemical dispersants is one common method of oil spill response, particularly in spills at sea. Dispersants work by preventing the formation of water-in-oil emulsifications that prevent the natural dispersion and biodegradation of oil in seawater [84]. However, the use of dispersants is often restricted by governments, limiting their use to open ocean scenarios away from sensitive habitats such as mangroves and coral reefs [85]. In at-sea incidents, dispersants have been demonstrated to be effective at preventing spilled oil from reaching the shore, eliminating the toxic effects to shoreline biota and obviating the difficult and

expensive cleaning associated with shoreline oiling [85]. Additionally, some research indicates that dispersants may be used on nearshore oil spills with a resultant toxicity lower than that of the spilled oil alone [86], and that current toxicological tests do not directly reflect environmental conditions, as the toxic components of dispersants are not found in high concentrations for extended periods of time in a marine oil spill response [87].

### **5.3.2 Skimmers and pumps**

Methods that offer physical removal and potential reclamation of spilled oil can be ecologically and financially attractive. Skimming and pumping generally involves containing spilled oil in a boom, taking it up with a skimmer head and pumping it into a storage container [75]. Containing booms can prevent oil from reaching the shoreline, preventing a significant increase in manpower, costs and ecological damage.

There are significant practical limitations to skimming and pumping of spilled oil. Skimming is considered appropriate only for very large quantities of oil [75]. Oil that is heavily contaminated with sediment, such as oil washed from a shoreline or in a high energy environment, can foul skimmers and pumps. Similarly, heavy oils, such as bunker oil and heavy crude, as well as weathered oil, may be too viscous to be successfully pumped [88]; in fact, even in ideal conditions heavy oils must be heated to be successfully pumped [89]. Additionally, containment booms may fail in heavy seas, due to either tidal conditions or heavy weather [79].

### **5.3.3 Mechanical scraping**

Once a shoreline has been oiled, mechanical removal of the oil is often the highest priority. Employing heavy earthmoving machinery, such as backhoes and front loaders, to physically remove oil and oiled sediment is commonly practiced in incidents of extensive shoreline oiling. In such cases, the spilled oil is concentrated into a designated area and loaded into a transport container [75].

The practical limitations to the use of heavy machinery can vary depending upon the circumstances. Shorelines must be accessible to the heavy machinery, and the access roads must be of a reasonable size and quality. If oil is spilled in a remote location, acquiring the machinery, as well as the skilled labour to operate it, may be difficult and time-consuming [77]. Mechanical removal is also a slow process, and the longer clean up takes, the greater the risk of long term impacts. Utilising a scraping and removal method can result in the loss of a significant quantity of the

natural substrate, and this removal may be compounded by the damage caused by the tracks of the machinery to result in a high degree of shoreline erosion [75].

### **5.3.4 Manual removal**

In the event of a small to medium sized spill, or if other response methods are too difficult, manual removal by teams of responders may be used. Manual removal methods are also frequently used in conjunction with other methods for intensive cleaning in localised areas or on portions of the shoreline inaccessible to other methods. In such circumstances, responders undertake cleaning tactics similar to those used in the heavy machinery aided response, albeit on a smaller scale: oil is collected into a localised concentration and then removed by small skid loaders or bagged and removed by boat or vehicle [75].

Water based techniques are also used to remove oil. Water that is heated to reduce the viscosity and increase the mobility of the spilled oil is applied by spray, and cold water (or steam) can be applied under high pressure in order to remove oil. This approach is mainly suited to spills in which oil is fouling hard surfaces, particularly if the oil is weathered or particularly viscous. Spraying can remove oil that has settled in cavities in rocks and from sheer rock faces that are inaccessible to other cleaning methods [75].

Any type of manual removal is very labour intensive, and the nature of the work exposes responders to significant hazards through the oil itself, cleaning agents or prevailing conditions. Manual methods also tend to be extremely labour intensive, slow and generate large quantities of waste. Sediment removal and erosion is another risk, and in remote areas, gathering the necessary number of responders may be problematic.

Furthermore, spraying requires machinery access in addition to a large number of people. Oil removed by spraying should be collected by a boom and skimmed, otherwise it will wash back into the ocean, furthering damaging nearshore biota or washing back onto the shoreline. Additionally, spraying the oil with high pressure can drive oil into the sediment or cause oil-in-water emulsions.

### **5.3.5 Bioremediation**

Studies of the utilisation of microbes as a biological form of oil spill remediation began in response to ZoBell's groundbreaking review of hydrocarbon-digesting bacteria, published in 1946 [90]. Since that time, considerable effort has been

invested to understand what factors enhance or inhibit bioremediation. This topic has deservedly warranted many comprehensive reviews that deal with the topic in much greater depth than can be achieved here; for examples see Atlas [21], Hoff [91], Leahy & Colwell [92], Mearns [93], Swannell et al. [94], Venosa [95] and Wantanabe et al. [96].

Bioremediation occurs when hydrocarbon-digesting bacteria and fungi break down the hydrocarbons in the spilled oil, thus decreasing the toxicity of spilled oil and reducing the potential for oil to penetrate into the sediments and contribute to re-oiling [61]. Such microbes exist in almost every environment studied, from salt to fresh water, from Arctic to tropical waters, as well as in terrestrial soils [21, 97]. While heavy crudes and fuel oils are more resistant to biodegradation than lighter distillates [92], a process known as co-oxidation, reviewed by Atlas [21], allows microbes to degrade hydrocarbon compounds that they would not normally affect, due to presence of a more readily available hydrocarbon substrate. For any such degradation process to occur, however, conditions for bacterial growth must be met and any limiting conditions alleviated. Important abiotic factors that limit biodegradation, identified and reviewed by Leahy & Colwell [92], include: temperature, oxygen level, nutrients and pH. Similarly, certain conditions of the spilled oil, such as its physical state (i.e. dispersed vs. emulsified) and its concentration can play a significant role in the degree to which it can be influenced by bioremediation [92]. Field and laboratory studies have shown that bioremediation can be enhanced by the addition of nutrients to further facilitate microbial growth, increasing the surface area of the spilled oil, increasing oxygen transport through soil tilling, or aeration and inoculating the oil spill with known hydrocarbon-degrading organisms [93].

In one of the few studies on oil spill bioremediation in Australia, Ramsay et al. [98] found that mangrove sediments are likely to facilitate bioremediation, as the organic rich environment may host a variety of hydrocarbon degrading microbes. However, the effectiveness of these native microbes may be limited by the relatively anoxic conditions of mangrove sediments. The sediments of oiled and unoiled mangrove stand plots were aerated and fertilisers added to some of the oiled plots. Aeration occurred over a period of four months, whereas fertilisation occurred soon after oiling and six months after oiling. The population levels of native hydrocarbon-digesting bacteria increased in response to both oiling and nutrient addition. Furthermore, the increases were up to 100 times greater than previously reported bacterial blooms in bioremediation trials on beaches. The authors suggest this increase resulted from

the bacteria rich mangrove sediments and the addition of fertilisers resolving the nutrient limited conditions.

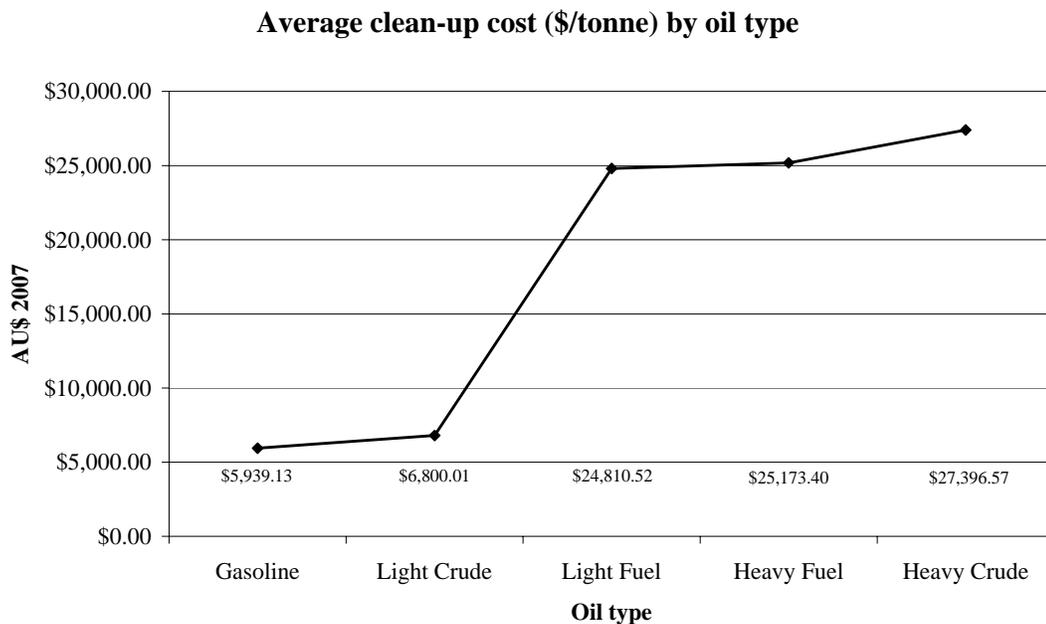
Inoculating spilled oil with bacteria has been shown to enhance hydrocarbon digestion, however such trials have been unable to resolve whether it is more effective to instead remedy conditions that tend to limit populations of naturally occurring hydrocarbon-digesting bacteria. Some studies have shown positive results from bacterial inoculation [57, 99-101], whereas others have failed to show any enhancement of bioremediation by such inoculations [10, 11, 95, 102-104], and that inoculation may inhibit the native bacteria that may be more suited to the local environment. Mearns [93] concludes that microbial inoculation is “neither necessary nor sufficient” to stimulate biodegradation of spilled oil, and that nutrients and oxygen are the limiting factors to bioremediation, not bacteria. Furthermore, introducing live bacterial cultures to remediate oil spills may invite risk to environmental damage greater than the oil spill. As previously mentioned (1.1), oil spills can result in phase shifts in which particularly sensitive organisms are killed off in very large numbers, whereas more tolerant organisms are allowed to disrupt previous balance and dominate the system [66-68]. Introducing an exotic bacterial species that is adapted to thrive in an oiled environment can be detrimental to the native bacteria in this stressed system.

In summary, despite some promising results bioremediation has limitations as strategy on which to rely following an oil spill. The Baffin Island Oil Spill (BIOS) experiment [105] revealed that while some oil spilled into an Arctic environment was thoroughly degraded by native bacteria, other patches of oil persisted for 20 years, appearing as “fresh as the day it was spilled”. Several factors may have influenced this persistence. As the effectiveness of bioremediation depends upon the ability for hydrocarbon degrading microbes to thrive, pristine environments may be relatively deficient in hydrocarbon digesting organisms [106]. Additionally, the abiotic factors, such as temperature, salinity and pH can influence the ability of bacteria to degrade hydrocarbons [92]. Even with normal microbial populations and ideal conditions, biodegradation is not a fast process. Areas of mangroves or beaches that remain oiled for a long period of time as bioremediation occurs may attract criticism from the public and result in economic losses. If bioremediation is employed in an unsuitable area, the results can be very long lasting [107], placing a premium on careful consideration of whether bioremediation is the best response method.

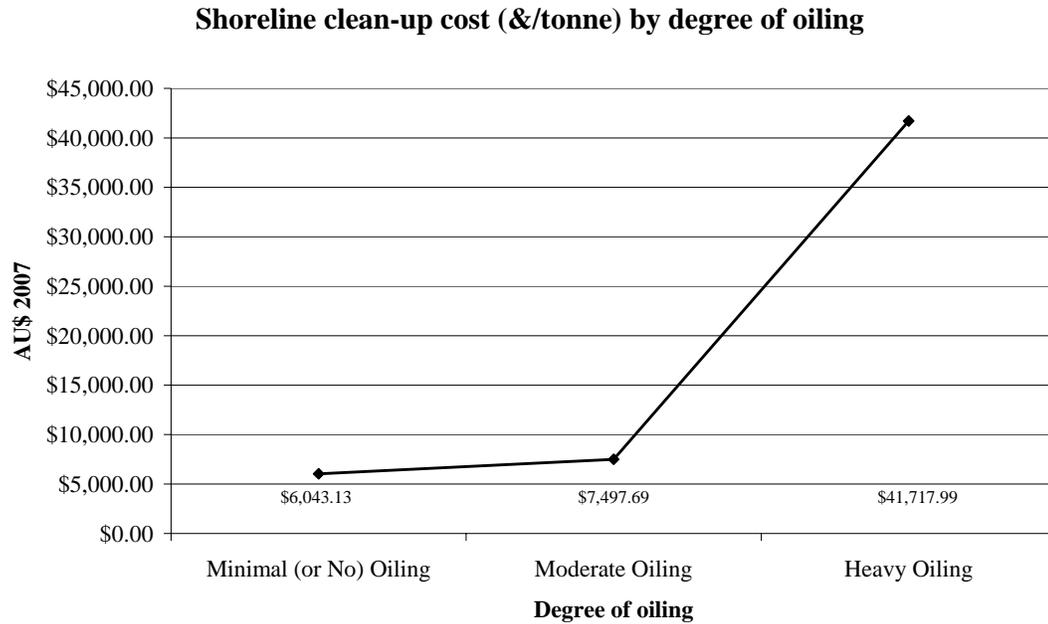
## 5.4 Costs of oil spill remediation

### 5.4.1 Monetary

Oil spill cleanup and remediation is a costly endeavour. The greatest costs are in the mobilisation of the oil spill response; therefore a small oil spill may cost nearly as much as a much greater one. Indeed, the response to the grounding of the *Peacock* in Far North Queensland resulted in over \$800,000 in costs despite no oil being spilled [77]. A number of factors contribute to the cost associated with oil spill remediation, the most important of which are the type of oil spilled and the location of the spill (Figs. 3 & 4) [108]. As mentioned above, crude oil and bunker oil are the most difficult oils to clean, as they contain a lower percentage of volatile components and are more viscous. Figure 5 shows the relative costs of several common response methods.

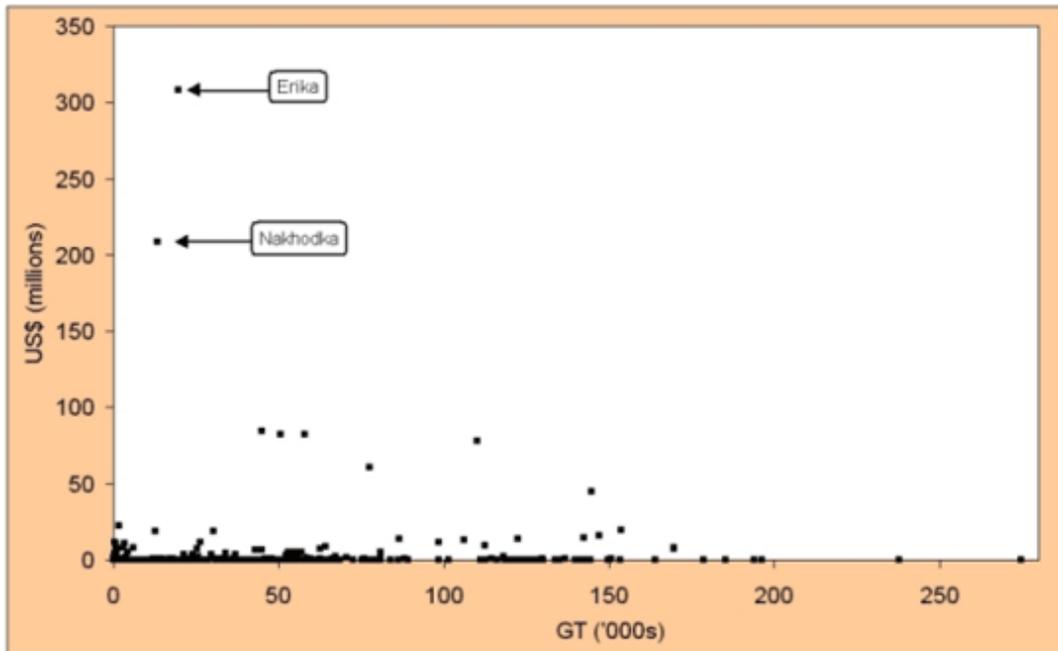


**Figure 3. Clean up cost increases with heavier types of oil. Costs are per tonne in AU\$ 2007. Adapted from Etkin 1999 [108].**



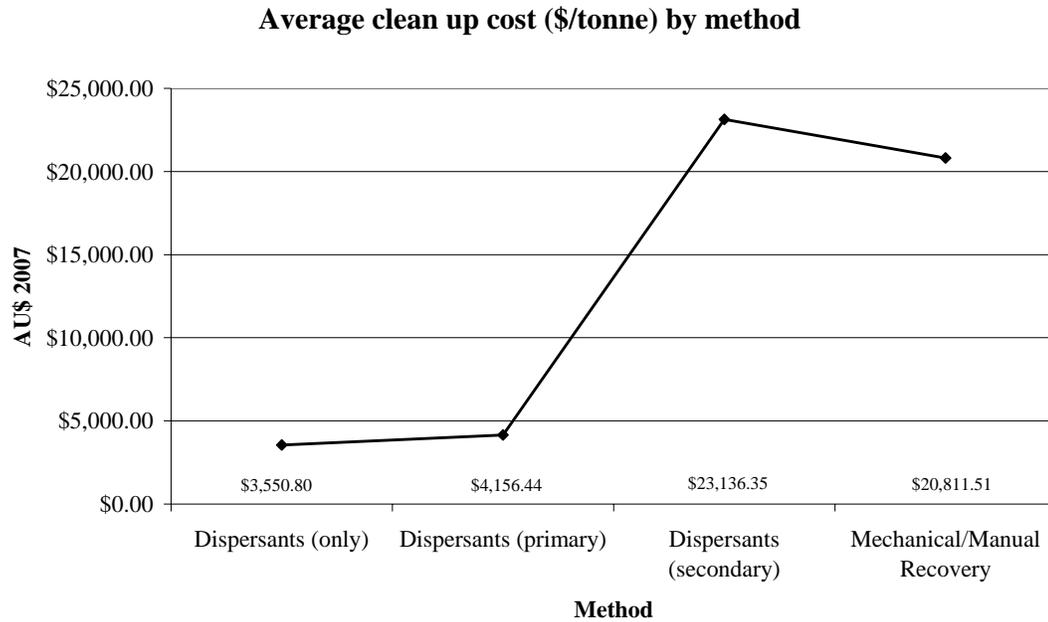
**Figure 4. The effects of the degree of shoreline oiling on the cost of cleaning. Prices are per tonne in AU\$ 2007. Adapted from Etkin 1999 [108].**

The second major factor contributing to the cost of oil spill cleanup is the location of the oil, in respect to the proximity of the coast. Oil spilled offshore tends to be easier and less expensive to clean, particularly when dispersants can be employed. One study estimated the average cost of offshore oil removal at \$7,350/tonne, whereas shoreline cleanup costs as much as \$147,000-\$294,000, or 2000-4000% more [85]. Similarly, the British Oil Spill Control Association (BOSCA) estimated the cost oil spill cleanup via dispersant treatment at \$174-\$350/tonne [85]. Additionally, oiled beaches can result in a loss of tourism revenue, cleaning payments to private boat owners and a number of other financial considerations. There is no apparent direct relationship between size of spill and cost of cleanup (Fig. 5).



**Figure 5. Cost of oil spill clean up in millions of US\$ per thousands of gross tonnes of oil. Size of spill does not play a direct role in cost of clean up. Figure from ITOPF [74].**

Manual removal methods, such as beach scraping, earth moving equipment, skimming and pumping, require sizable investments in manpower and machinery. Often, a great deal of the manpower needed is supplied by volunteers eager to help rectify an environmental disaster, such as the volunteer response that occurred to the *Prestige* spill [109]. However, untrained volunteers need trained supervisors and any heavy equipment needs trained operators. Furthermore, the costs involved in securing the equipment and boats necessary quickly mounts [77]. Etkin's [108] oil spill cost analysis indicates that a manual cleanup response to a spill of heavy crude that heavily oils a shoreline may cost as much as \$27,460 per tonne in Australia. Of course, the per tonnage estimate does not consider the costs that may be incurred if the spill occurs in a remote, difficult to access shoreline or if the response time is hindered by distance or conditions.



**Figure 5. The average cost per tonne of cleaning oil based upon the response method in AU\$ 2007. Adapted from Etkin 1999 [108].**

The oiled waste generated by methods such as manual removal, heavy equipment removal and some forms of bioremediation must be dealt with in a manner that guarantees against further contamination of soil or groundwater. One such method, land-farming, is a process built on the same premises as bioremediation [110]. Oiled sediment is transported to a remediation facility where it is added to containment systems lined with impenetrable membranes that prevent outside contamination. Similar to shoreline bioremediation, the contaminated sediment is then cleaned by soil bacteria, which is further enhanced by treatment with nutrients and tilling. This method is a fairly cheap way to deal with the waste generated by extensive clean up procedures, with estimates of \$30-\$70 per tonne of sediment treated [110].

The costs incurred if a bioremediation response is adopted can be extremely variable. If, as in the case of the *Solar I* spill in the Philippines [75], the oil is left alone to degrade naturally, the cost is relatively modest. Immediate and long-term monitoring contribute the bulk of expenses in such a response. However, if a bioremediation response involving the application of nutrients or bacterial inoculations is adopted, response costs could begin to reach the level of manual responses.

## 5.4.2 Environmental

The environmental costs of oil spill response are potentially greater than the monetary costs, therefore, consideration must be given to the negative consequences and limitations of remediation techniques to ensure the countermeasure utilised does not cause more damage than that already caused by the spill. Table 1 [81] details a variety of at-sea and shoreline oil spill remediation methods and the potential threat each method poses to the flora and fauna. All methods have some negative impact, and this must be weighed against the impact of the oil spill itself. For example, in response to the *Exxon Valdez* spill, contaminated brown algae (*Fucus* sp.) were intensively cleaned by volunteer workers using manual cleanup methods including hot, pressurised water spray. This was later found to have resulted in more severe and persistent damage to the algae than in areas that were untreated [65, 82, 93]. Such methods have also been shown to result in massive deaths to shoreline biota [79]. Similarly, the foot traffic and the use of heavy machinery involved in manual recovery methods can drive oil into the sediment, reducing the effectiveness of removal efforts and increasing both the persistence of oil and the chance of re-oiling [93].

Manual removal, including skimming, scraping and heavy machinery facilitated cleanup, also generate extensive amounts of waste. This oiled waste must then be disposed of, and is commonly done so in large trenches slightly inshore from the spill, resulting in persistent toxic collections of oiled waste, a process known as land farming [110].

Dispersants also have their drawbacks. The greatest practical limitation is that dispersants are relatively ineffective in dealing with heavy or weathered oils [81], which are likely to be encountered in a shoreline spill response. Dispersant toxicity is the greatest ecological limitation. Early use of dispersants, such as in the response to the *Torrey Canyon* spill in 1967 resulted in massive mortality to marine life, as the toxicities of the chemicals were not fully understood prior to use [85, 111-113]. These early dispersants were later found to be profoundly toxic to aquatic organisms [112], and while modern formulations have been shown to be less toxic [113, 114], responders remain cautious. Despite a decrease in toxicity, modern dispersants are still considered moderately toxic to some aquatic organisms [114, 115] and may inhibit microbial degradation processes [116]. This toxicity may be exacerbated in responses to heavy or weathered oils, which may require more concentrated application rates thus subjecting organisms to elevated concentrations of toxic

components [113]. Additionally, the increase in the amount of oil in the water column from successful dispersion increases the exposure of aquatic organisms to toxic compounds in the oil [117, 118].

Additionally, despite past use in shoreline clean-ups, dispersants serve better in spills at sea. They function to break up oil slicks and allow the oil to disperse into the water column, to a depth of greater than 10 meters [84], which can result in extremely high concentrations of oil-dispersant mixture in shallow nearshore environments, exacerbating toxic effects [117, 118]. This increased contact with the seafloor may also result in the absorption of the dispersed oil into the sediment, contributing to future re-oiling [117, 118].

Bioremediation techniques can have a wide variety of environmental impacts. In response to the 2006 *Solar 1* spill in the Philippines, which contaminated mangrove areas, the decision was made to leave the spill alone, rather than further stress the environment [75]. It remains to be seen if the environment can fully recover from this incident, though mangrove areas have been previously shown to display a high potential for bioremediation [98]. Similarly, spilled oil has been shown to naturally disperse in high energy environments, allowing for minimal further impact on the spill site [119], though in the case of large spills, leaving the oil can result in long term contamination [107]. One study found that oil spilled in an Arctic environment persisted for 20 years and appeared as fresh as when it was spilled [105].

Nutrient application and microbial inoculation with foreign microbes can introduce environmental problems as well. Nutrient application has thresholds below which the application is ineffective and above which has been shown to actually inhibit bacterial growth [120, 121]. Furthermore, adding high levels of nutrients can result in nutrient toxicity and future eutrophication [122]. In fact, in some waterways with high levels of anthropogenic pollution, nutrients may not be the limiting factor to bioremediation and any application would be redundant [103]. Similarly, the microbial population may not be a limiting factor to bioremediation and adding exotic bacteria may inhibit the growth of native populations that are more suited to the local conditions [103].

**Table 1. Countermeasures available to marine spill responders and some of their ecological impacts. Asterisks denote actions requiring special approval. Modified from Mearns 2002 [81].**

Shoreline Cleaning Method	Ecological Impact
Natural bioremediation	Slow, smothering and toxicity to shoreline biota
Manual removal	Damaging foot traffic, generates large quantities of waste
Mechanical removal	Physical shoreline damage, generates large quantities of waste
Skimming/pumping	Fuel consumption, foot traffic
Containment booms	Oil in undertow water, chains disturb sediment and seagrass beds
Sediment tilling	Physical damage to sediment, beach erosion
Burning	High biota mortality, smoke, unsightly residues
Ambient temperature washing	
Low pressure	Nearshore dispersal of oil
High pressure	Nearshore dispersal of oil, mortality to biota, risk of emulsions
Warm/hot water washing	Nearshore dispersal of oil, mortality to biota, risk of emulsions
Sand and slurry blasting	Nearshore dispersal of oil, mortality to biota, risk of emulsions
Chemical countermeasures	Toxicity, nearshore dispersal, shoreline removal, oiling of sediment
Bioremediation	
Nutrient addition	Nutrient and/or metabolite toxicity, eutrophication
Microbial inoculation	Metabolite toxicity, inhibition of native hydrocarbon digesting bacteria

## 6 Appendix B: Tabulation of literature review content in terms of the original tender.

The following table set out the objectives listed in the original proposal using the same layout as contained in the original proposal (left hand column) against the location of information relating to that in the report as it is presently structured (right hand column).

I.	Compile database of recent oil spills	Comprehensive databases available at <a href="http://www.itopf.com/">http://www.itopf.com/</a> and <a href="http://www.cedre.fr/index_gb.html">http://www.cedre.fr/index_gb.html</a>
	a. Country	See <a href="http://www.itopf.com/">http://www.itopf.com/</a> and <a href="http://www.cedre.fr/index_gb.html">http://www.cedre.fr/index_gb.html</a>
	b. Environmental classification of effected area (mangroves, beach, ice, open water, etc.)	See <a href="http://www.itopf.com/">http://www.itopf.com/</a> and <a href="http://www.cedre.fr/index_gb.html">http://www.cedre.fr/index_gb.html</a>
	c. Agencies involved in cleaning	See <a href="http://www.itopf.com/">http://www.itopf.com/</a> and <a href="http://www.cedre.fr/index_gb.html">http://www.cedre.fr/index_gb.html</a>
	i. Government agencies	Agencies are given at <a href="http://www.itopf.com/">http://www.itopf.com/</a> and <a href="http://www.cedre.fr/index_gb.html">http://www.cedre.fr/index_gb.html</a> when available
	ii. Commercial agencies	Agencies are given at <a href="http://www.itopf.com/">http://www.itopf.com/</a> and <a href="http://www.cedre.fr/index_gb.html">http://www.cedre.fr/index_gb.html</a> when available
	iii. Universities	Agencies are given at <a href="http://www.itopf.com/">http://www.itopf.com/</a> and <a href="http://www.cedre.fr/index_gb.html">http://www.cedre.fr/index_gb.html</a> when available
	iv. Environmental groups	Agencies are given at <a href="http://www.itopf.com/">http://www.itopf.com/</a> and <a href="http://www.cedre.fr/index_gb.html">http://www.cedre.fr/index_gb.html</a> when available
	d. Methods used for cleaning	Brief description of cleaning methods are located in § <a href="#">5.3 Common methods of oil spill remediation</a>
	e. Cost of cleaning	Costs of cleaning are discussed in § <a href="#">5.4 Costs of oil spill remediation</a>
	i. Monetary	Monetary costs of cleaning are discussed in § <a href="#">5.4.1 Monetary</a>
	ii. Time	Time spent cleaning is not discussed as this is dependant upon conditions particular to each spill.
	iii. Environmental degradation	Environmental costs of cleaning are discussed in § <a href="#">5.4.2 Environmental</a>
II.	Effectiveness of cleaning	Section discusses effectiveness of specific cleaning methods; effectiveness of cleaning individual spills may not be apparent for decades.
III.	Characterise current biodiesel research	Articles and research reports published in international journals are discussed in § <a href="#">2.2 Trials of biodiesel in oil spill remediation</a>
	a. United States	See § <a href="#">2.2 Trials of biodiesel in oil spill remediation</a>
	b. Europe	See § <a href="#">2.2 Trials of biodiesel in oil spill remediation</a>
	c. Australia	See § <a href="#">2.2 Trials of biodiesel in oil spill remediation</a>
	d. Asia	See § <a href="#">2.2 Trials of biodiesel in oil spill remediation</a>
IV.	Construct database of biodiesel products currently available and likely to be available in the near future	Database of U.S. EPA approved products available at: <a href="http://www.epa.gov/emergencies/content/ncp/product_schedule.htm">http://www.epa.gov/emergencies/content/ncp/product_schedule.htm</a>

a. Identify products	See: <a href="http://www.epa.gov/emergencies/content/ncp/product_schedule.htm">http://www.epa.gov/emergencies/content/ncp/product_schedule.htm</a>
i. Products previously used	Articles and research reports using biodiesel for cleaning oil spills are discussed in § <a href="#">2.2 Trials of biodiesel in oil spill remediation</a>
ii. Products proposed for use by international research	See: <a href="http://www.epa.gov/emergencies/content/ncp/product_schedule.htm">http://www.epa.gov/emergencies/content/ncp/product_schedule.htm</a>
iii. Products recommended by manufacturers	Section 3.3.2.2 discusses Cytosol, currently the only biodiesel product produced specifically for oil spill cleaning.
iv. Costs from manufacturers/suppliers	Requests for costs were not provided by manufacturers; will depend upon availability and quantity.
b. Availability	Biodiesel availability is discussed in § <a href="#">2.1.2.5 Biodiesel production and availability in Australia</a>
i. Manufacturers	See § <a href="#">2.1.2.5 Biodiesel production and availability in Australia</a>
ii. Universities	Production from non-commercial sources was not investigated due to the volume necessary for this application
iii. United States	International production was not investigated due to the costs and time sensitivity associated with oil spill response.
iv. Europe	International production was not investigated due to the costs and time sensitivity associated with oil spill response.
v. Australasia	See § <a href="#">2.1.2.5 Biodiesel production and availability in Australia</a>
c. Compile physical and chemical data (i.e. human and animal toxicities)	Toxicity is discussed in § <a href="#">2.1.2.4 Toxicity</a>
i. MSDS	Material safety data sheets for two biodiesels are attached; See § <a href="#">7 Appendix C: Material Safety Data Sheets (MSDS) for biodiesels and biodiesel based products</a>
ii. Research reports	See § <a href="#">2.1.2.4 Toxicity</a>
iii. Medical/research reports on biodiesel exposure in humans, plants, animals	See § <a href="#">2.1.2.4 Toxicity</a>
d. Assessment of response of products to abiotic variables.	The response of biodiesel to abiotic variables is discussed in a limited fashion in § <a href="#">2.1.2.3 Biodegradability</a>
i. Temperature	No data is currently available on the response of the biodiesels tested to temperature.
ii. Solar radiation	No data is currently available on the response of the biodiesels tested to solar radiation.
iii. Agitation with water	No data is currently available on the response of the biodiesels tested to agitation with water.
iv. Salinity	No data is currently available on the response of the biodiesels tested to salinity.

## **7 Appendix C: Material Safety Data Sheets (MSDS) for biodiesels and biodiesel based products**

## 7.1 Palm oil biodiesel



NATURAL FUELS  
AUSTRALIA LIMITED

BIODIESEL

Revision:01  
Dated 27<sup>th</sup> January 2008  
Page 1 of 4

### Material Safety Data Sheet

NON-HAZARDOUS ACCORDING TO THE CRITERIA OF WORKSAFE AUSTRALIA (NOHSC)

#### Section 1 Identification of the Preparation and the Company

##### 1.1 Identification of the preparation

Product name: Biodiesel  
Other Names: Methyl ester  
Product code: None  
Intended use: Automotive and heating fuel

##### 1.2 Identification of the Company

Manufacturer	Natural Fuels Australia Ltd
Address	800 Berrimah Road East Arm NT Australia 0828
Telephone	+61 8 8999 9799
Facsimile	+61 8 8947 1452
Email	jgray@naturalfuel.com
Australian emergency phone number	Poisons Information Centre. Phone (eg Australia 13 1126; New Zealand 0800 764 766).

#### Section 2 Hazard Identification

NON-HAZARDOUS SUBSTANCE: The product is not classified as hazardous according to the criteria of Worksafe Australia (NOHSC). It is not a Scheduled Poison. It is not a dangerous good. It is a Class C2 Combustible Liquid.  
RISK TO PERSONNEL: This product is derived from edible vegetable oils and is not expected to pose a hazard to personnel during normal handling or use.  
RISK TO THE ENVIRONMENT: The product is believed to be readily biodegradable. However, due to its oily nature, large spills could potentially create an oily film on water which might have a localised, adverse effect on aquatic organisms.  
RISK PHRASES:None SAFETY PHRASES: None

#### Section 3 Composition/Information on Ingredients

The product is an edible vegetable oil derivative which contains no hazardous ingredients at concentrations above the concentration cut-offs specified by Worksafe Australia (NOHSC).

Name	CAS Number	Concentration
Fatty acid methyl esters derived from vegetable oil		
Methyl laurate	111-82-0	<55%
Methyl myristate	124-10-7	<20%
Methyl linoleate	Not available	<72%
Methyl oleate	111-62-9	5-85%
Methyl palmitate	112-39-0	4-30%
Methyl ester	Not available	3-6%

#### Section 4 First-aid Measures

EYES: If in eyes, hold eyelids apart and flush the eye continuously with running water for 15 minutes. Seek medical attention if any irritation or discomfort persists.  
SKIN: Remove contaminated clothing. Rinse the affected area with water then wash thoroughly with soap and water. Use water alone, if soap is unavailable. Seek medical attention if any soreness or inflammation of the skin persists or develops later. Launder affected clothing before re-use.



**INGESTION:** Ingestion is not expected to result in adverse effects and first aid is unlikely to be required. However, if large amounts are ingested, give about 250mL (2 glasses) of water to drink and seek medical attention, as a precautionary measure. For advice, contact a Poisons Information Centre (phone eg Australia 131 126; New Zealand 0800 764 766).  
**INHALATION:** The product has such low volatility that inhalation is not a hazard of normal use. If someone were exposed to vapours, for example if the product were heated strongly, then remove the person to fresh air. Keep warm and at rest. Further first aid is unlikely to be required but medical attention should be sought if any symptoms persist.  
**ADVICE TO DOCTOR:** Treat symptomatically

#### Section 5 Fire-fighting Measures

**EXTINGUISHING MEDIA:** Extinguish using foam, carbon dioxide or dry chemical extinguishers. Water may be used to cool fire exposed containers but is not suitable for fire fighting.  
**HAZARDOUS COMBUSTION PRODUCTS:** May evolve carbon monoxide or dioxide if heated to decomposition or burned in a fire situation.  
**PRECAUTIONS:** Fire fighters should wear self-contained breathing apparatus in a fire situation.

#### Section 6 Accidental Release Measures

Remove unnecessary personnel from the affected area. Wear safety glasses or goggles and oil impermeable gloves (PVC, nitrile or neoprene are suitable). If possible, dam the spill. Cover with an absorbent such as earth, sand or a commercial oil absorber. Sweep up and collect in sealable containers. Dispose to approved land-fill. Do not allow to enter drains or water courses

#### Section 7 Handling and Storage

Avoid any contact with the skin or eyes.  
Product is a Class C2 Combustible liquid.  
Store out of direct sunlight in a cool well ventilated. Higher temperatures may cause pressure build up inside containers. Storage area should be designated no smoking. Keep away from all sources of ignition. Protect containers against physical damage. Avoid contact with strong oxidising agents.

#### Section 8 Exposure Controls / Personal Protection

**EXPOSURE STANDARDS:** Exposure Standards have not been allocated to this product. Information for ingredients is:  
Oil mist E.S. TWA: 5mg/m<sup>3</sup>

Exposure standard represents the airborne concentration of a particular substance in the worker's breathing zone, exposure to which, according to current knowledge, should not cause adverse health effects nor cause undue discomfort to nearly all workers. The exposure standard can be of three forms; time-weighted average (TWA), peak, or short term exposure limit (STEL).

**BIOLOGICAL LIMIT VALUES:** None allocated.

**ENGINEERING CONTROLS:** Good, general ventilation should be sufficient for all anticipated use and handling of this product. However, sufficient ventilation must be provided to keep airborne levels below the exposure limit. Where vapours or mists are generated, particularly in enclosed areas, and natural ventilation is inadequate, a local exhaust ventilation system is required. Refer to AS 1940 - The storage and handling of flammable and combustible liquids and AS 2430 - Explosive gas atmospheres for further information concerning ventilation requirements.

**PERSONAL PROTECTION:** Requirements are dependant on working conditions, quantity of product in use and method of application. For minor use safety goggles and nitrile, neoprene, polyvinyl chloride (PVC) or natural rubber gloves may be sufficient. If large quantities are in use; chemical resistant safety goggles, gloves or gauntlets and overalls. A half face respirator with organic solvent vapour filter may be required if the product is heated in a confined or poorly ventilated area. N.B. TAKE THE LIMITS OF ABSORPTION CAPACITY INTO ACCOUNT. CHANGE FILTERS REGULARLY.



**Section 9 Physical and Chemical Properties**

Appearance	Oily, clear liquid.
Odour	Vegetable oil
Colour	Yellow
Solubility	Not miscible
Partition coefficient n-octanol / water	>3 (Log Kow)
Viscosity (mm <sup>2</sup> /s)	4.09 at 40 °C
Ph: 1% Solution	Not pertinent
Boiling point	>150°C
Flash point	170°C
Flammable Limits	No data available
Vapour pressure	Negligible
Evaporation rate	Negligible
Specific gravity	0.88

**Section 10 Stability and Reactivity**

CHEMICAL STABILITY: Stable  
CONDITIONS TO AVOID: Product will burn if heated strongly. Avoid all sources of ignition such as open flames, sparks, hot surfaces or burning cigarettes.  
INCOMPATIBLE MATERIALS: The product may react with strong oxidising agents such as liquid or powdered chlorine.  
HAZARDOUS DECOMPOSITION PRODUCTS: Carbon monoxide and carbon dioxide.  
HAZARDOUS REACTIONS: None known

**Section 11 Toxicological Information**

HEALTH HAZARDS ACUTE  
INGESTION: Low hazard. The compounds contained in this product have been approved as food additives by the US Food and Drug Administration.  
EYE: Liquid and high vapour concentration may cause some irritation and watering of the eyes but no eye injury is expected to result from contact.  
SKIN: Low hazard. Although methyl palmitate and stearate have been shown to be irritating to rabbit skin, esters of fatty acids (C<sub>6</sub> to C<sub>18</sub>) have not been shown to have any irritating effects in humans.  
INHALATION: The product has negligible volatility at normal temperatures so inhalation is considered to be extremely unlikely during any anticipated use of the product. If generated, oil mists are irritating to the respiratory system.  
HEALTH HAZARDS CHRONIC  
Ingestion is the only route of entry into the body. No chronic health hazards have been identified.  
Fatty acid esters (palmitic, oleic, stearic) LD50 (oral, rat): >5000mg/Kg, LD50 (skin, rat): >5000mg/Kg.

**Section 12 Ecological Information**

ECOTOXICITY: Product has the potential to form an oily film on water and this physical property may cause harm to aquatic organisms but it is not considered to be toxic.  
MOBILITY: Volatilization from moist soil surfaces is expected to be an important fate process given an estimated Henry's Law constant of 0.014 atm-cu m/mole. However, adsorption to soil is expected to attenuate volatilization. It is not expected to volatilize from dry soil surfaces based upon a vapor pressure of 6.3X10<sup>-6</sup> mm Hg.  
BIODEGRADATION: Product is expected to rapidly biodegrade in aerobic soils as suggested by the rapid biodegradation of structurally similar long-chain fatty acid esters

**Section 13 Disposal Considerations**

Dispose by controlled incineration or to approved land-fill. Do not allow to enter drains or waterways.



#### Section 14 Transport Information

This product is a Class C2 Combustible Liquid. It is not classified as a dangerous good according to the Australian Code for the Transportation of Dangerous Goods by Road and Rail (ADG Code).

UN Number: Not applicable  
Proper shipping name: Not applicable  
DG Class: None  
HazChem code: None  
Packing group: Not applicable

#### Section 15 Regulatory Information

Product is not a Scheduled Poison according to the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).

#### Section 16 Further Information

Date of Preparation: 27/01/06  
Prepared by: C M Ferrins, Consultant Industrial Hygienist

##### REFERENCES

1. List of Designated Hazardous Substances [NOHSC: 10005(1999)]
2. National Code of Practice for the Preparation of Material Safety Data Sheets 2<sup>nd</sup> Edition [NOHSC: 2011(2003)]
3. Exposure Standards for Atmospheric Contaminants in the Occupational Environment [NOHSC: 1003(1995)] and subsequent amendments
4. Australian Code for the Transportation of Dangerous Goods by Road and Rail (ADG Code), 6th Edition, 1998
5. Australian/New Zealand Standard AS/NZS 1715, Selection, Use and Maintenance of Respiratory Protective Devices
6. Australian/New Zealand Standard AS/NZS 1716, Respiratory Protective Devices
7. Australian/New Zealand Standard AS/NZS 1337- Eye Protectors for Industrial Applications
8. Australian/New Zealand Standard AS/NZS 2161 Occupational protective gloves- Selection, use and maintenance.

##### ABBREVIATIONS

LC50 Lethal dose for 50% of test population, by inhalation.  
LDLo Lowest documented lethal dose  
LD50 Lethal dose for 50% of test population, by ingestion or skin contact  
TDLo Lowest published toxic dose

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Revision Number: 001  
Dated 27/01/2006



<b>Identification</b>	<b>Biodiesel</b>	
<b>Chemical Identity &amp; Characteristics</b>	Methyl Ester : Clear, yellow, oily liquid.	
<b>Compliance</b>	Australian Fuel Quality (Biodiesel) Determination 2003 European Biodiesel Standard EN 14214 US Biodiesel Standard ASTM D6751	
<b>Recommended Applications</b>	<ul style="list-style-type: none"> <li>&gt; Automotive Fuel</li> <li>&gt; Marine Fuel</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Stationary Generator Fuel</li> <li>&gt; Industrial Equipment Fuel</li> </ul>
<b>Transportation and Regulation</b>	Refer to Material Safety Data Sheet	

Parameter	Method	Units	Australian Fuel Quality (Biodiesel) Determination 2003	Typical Value
Ester Content	EN 14103	% (m/m)	≥ 96.5	97
Distillation T90	ASTM D1160	°C	≤ 360	355
Cetano Number	ASTM D613		≥ 51.0	66
Density @15°C	ASTM D1298	kg / m <sup>3</sup>	860 - 890	875
Viscosity @ 40° C	ASTM D445	mm <sup>2</sup> / s	3.5 – 5.0	4.5
Flashpoint	ASTM D93	°C	≥ 120	174
Copper Strip Corrosion (3hrs @50°C)	ASTM D130		Class 1 maximum	Class 1a
Acid Value	ASTM D664	mgKOH / g	≤ 0.8	TBD
Total Contamination	ASTM D5452	mg / kg	≤ 24	≤ 10
Oxidation Stability** @ 110°C	EN 14112	hrs	≥ 8	7.5
Sulfur	ASTM D5453	mg / kg	≤ 10	0.04
Sulfated Ash	ASTM D874	% mass	≤ 0.02	0.002
Carbon Residue on 100% distillation	ASTM D4530	% mass	≤ 0.05	0.004
Water and Sediment	ASTM D2709	% vol	≤ 0.05	<0.05
Phosphorus	ASTM D4951	mg / kg	≤ 10	≤ 1
Free glycerol	ASTM D6584	% mass	≤ 0.02	≤ 0.01
Total glycerol	ASTM D6584	% mass	≤ 0.25	≤ 0.1
Metals – Group I (Na, K)	ASTM D4951	mg / kg	≤ 5	≤ 1
Metals – Group II (Ca, Mg)	ASTM D4951	mg / kg	≤ 5	≤ 1
Methanol Content	EN 14110	% (m/m)	≤ 0.2	≤ 1
Cloud Point**	ASTM 2500	°C	Summer Winter	<+14 <+6
Cold Filtering Plugging Point**	IP 309	°C	Summer Winter	<+10 <+3
Filter Blocking Tendency <sup>#</sup>	IP 387		2.0	< 1.2
Calorific Value, (AS1038.5)	ASTM D240	MJ / kg		40

\*\* May be modified with additives

<sup>#</sup> Test a requirement of the Australian Fuel (Automotive Diesel) Determination 2001



SENT TO 6105 05/09/07. MID.

## ANALYTICAL REPORT

Client's Name: Natural Fuel Darwin Sample No.: 002107  
 Product: B100 Biodiesel Client Ref. No.: N/A  
 Source: 07F08A Job No.: 309870911L  
 Type of Sample: Not stated Date Sampled: 08/09/07-09:35  
 Container: 2 x 1 Litre Date Received: 05/09/2007-09:10  
 Remarks: Quality Verification Date Reported: 05/09/2007-20:39

Results relate only to sample(s) received. The above sample was tested and the following results were obtained:

Test	Test Method	Unit	Min	Max	Result	Pass/Fail
Particulate Contamination	ASTM D8452	mg/kg		24	9.9	PASS
Density @ 15 Deg C	ASTM D1298	kg/m <sup>3</sup>	880	890	875.7	PASS
Kinematic Viscosity @ 40 Deg C	ASTM D445	mm <sup>2</sup> /s	3.5	5.0	4.349	PASS
Water Content by KF	ASTM D4028	ppm			52.80	PASS
Water Content	EN 14103	% m/m	95.5		57.60	PASS
Methanol Content	EN 14110	% m/m		<0.20	<0.01	PASS
Free Glycerol	ASTM D8584	mass %		0.020	0.005	PASS
Total Glycerol	ASTM D8584	mass %		0.25	0.097	PASS
Total Acid Number (TAN)	ASTM D864	mg KOH/g		0.80	0.488	PASS
Sulphated Ash	ASTM D874	mass %		0.020	0.009	PASS
Carbon Residue on 100% distillation	ASTM D4530	mass %		0.050	0.010	PASS
Cetane Number	ASTM D613		51		95.0	PASS
Copper Corrosion, 3 Hrs @ 60 Deg C	ASTM D130	Grade		1	1a	PASS
Flash Point	ASTM D83	°C	120		172	PASS
Accelerated Oxidation Stability	EN14112	Hours	6		0.15-	PASS
Sulphur	ASTM D8453	mg/kg		10	1.090	PASS
Distillation T80	ASTM D1186	°C		380	348.0	PASS
Phosphorus	ASTM D4951	mg/kg		10	<1	PASS
Group I Elements						
Sodium	ASTM D4951	mg/kg		45	1.00	
Potassium	ASTM D4951	mg/kg		45	<1	
Total Group I Elements		mg/kg		Total 45	<1	PASS
Group II Elements						
Calcium	ASTM D4951	mg/kg		45	<1	
Magnesium	ASTM D4951	mg/kg		45	<1	
Total Group II Elements		mg/kg		Total 45	<1	PASS
Filter Blocking Tendency	IP387				Report	
Cloud Point	ASTM D2500	°C			Report	12.80
Cold Filter Plugging Point	ASTM D6371	°C			Report	+10

For: SGS Australia Pty. Ltd.

ROSEMIE MARCELO  
 QC&C Quality Chemist

Precision parameters apply in the determination of the above results. Also, refer to ASTM D-3244-07; IP 367/86; ISO 4209:1992 and Appendix E of IP Standard Methods for Analysis and Testing for utilisation of test data to determine conformance with specifications.

All figures are certified and reported in accordance with our General Conditions of Business unless otherwise agreed in a written contract.

SGS Australia Pty. Ltd. | 4129 Wattle Road, P.O. Box 39780 Wattle, Northern Territory 0821, Australia (+61 (8) 8947 4700 ) +61 (8) 8947 4711  
 Member of the SGS Group (Société Générale de Surveillance)

## 7.2 Coconut biodiesel

**CHEMREZ**  
TECHNOLOGIES

# Material Safety Data Sheet

CHE-AA-001

Date of First Issue: April 4, 2003  
Rev.No. 1  
Rev Date: November 5, 2004

Page 1 of 5

## Fil-Ester 618

CAS NO. 111-82-0

### 1. Chemical Product

Chemical Name & Synonyms

Coconut Oil Methyl Ester

Cocomethyl Ester

Trade Name

**FIL-Ester 618**

Chemical Family

Fatty Acid Methyl Ester

Formula

R-COO-CH<sub>3</sub>

### 2. Composition / Information on Ingredients

This product contains no hazardous materials. Fil-Ester 618 contains a variety of fatty acid methyl esters with carbon chain lengths varying between C<sub>16</sub> to C<sub>18,2</sub>, where the 16 and 18 carbon chains are the most common.

### 3. Hazards Identification

Potential Health Effects

INHALATION

**Threshold Limit Value (TLV):**

**Negligible** - Unless heated to produce vapors. Vapors or finely misted materials may irritate the mucous membranes and cause irritation, dizziness, and nausea. Remove to fresh air.

EYE CONTACT

May cause irritation. Irrigate eye with water for at least 15 to 20 minutes. Seek medical attention if symptoms persist.

SKIN CONTACT

Prolonged or repeated contact is not likely to cause significant skin irritation. Material is sometimes encountered at elevated temperatures. Thermal burns are possible.

Chemrez Technologies, Inc. 65 Industria St., Bagumbayan, Quezon City, 1110 Metro Manila, Philippines  
Tel.: (632) 635 0680 Fax: (632) 635 0703 E-mail: info@chemrez.com.ph Website: www.chemrez.com

10/11/2007

INGESTION

No hazards anticipated from ingestion incidental to industrial exposure.

**4. First Aid Measures**

EYES

Irrigate eyes with a heavy stream of water for at least 15 to 20 minutes.

SKIN

Wash exposed areas of the body with soap and water.

INHALATION

Remove from the area of exposure, seek medical attention if symptoms persist.

INGESTION

Give 1 or 2 glasses of water to drink. If gastro-intestinal symptoms develop, consult medical personnel. (Never give anything by mouth to an unconscious person.)

**5. Fire Fighting Measures**

Flash Point

142 °C

Flammability limits

none known

---

EXTINGUISHING MEDIA

Dry chemical, foam, halon, CO<sub>2</sub> water spray (fog). Water stream may splash the burning liquid and spread fire.

SPECIAL FIRE FIGHTING PROCEDURES

Use water spray to cool drums exposed to fire.

10/11/2007

#### UNUSUAL FIRE AND EXPLOSION HAZARDS

Oil soaked rags can cause spontaneous combustion if not handled properly. Before disposal, wash rags with soap and water and dry in well-ventilated area. Firefighters should use self-contained breathing apparatus to avoid exposure to smoke and vapor.

#### 6. Accidental Release Measures Spill Clean-up Procedures

Remove sources of ignition, contain spill to smallest area possible. Stop leak if possible. Pick-up small spills with absorbent materials such as paper towels, "oil dry", sand or dirt. Recover large spills for salvage or disposal. Wash hard surfaces with safety solvent or detergent to remove remaining oil film. Greasy nature will result in a slippery surface.

#### 7. Handling and Storage

Store in closed containers between 10°C (50°F) and 50°C (120°F).  
Keep away from oxidizing agents, excessive heat, and ignition sources.  
Store and use in well ventilated areas.  
Do not store or use near heat, spark, or flame, store out of sun.  
Do not puncture, drag, or slide the container.  
Drum is not a pressure vessel; never use pressure to empty.

#### 8. Exposure Control / Personal Protection

##### RESPIRATORY

If vapor or mist are generated, wear a NIOSH approved organic vapor / mist respirator.

##### PROTECTIVE CLOTHING

Safety glasses, goggles, or face shield recommended to protect eyes from mists or splashing. PVC coated gloves recommended to prevent skin contact.

10/11/2007

OTHERS

Employees must practice good personal hygiene, washing exposed areas of skin several times daily and laundering contaminated clothing before re-use.

**9. Physical and Chemical Properties**

Appearance and odor	clear yellow liquid, mild odor
Boiling point, 760mmHg	$\geq 200^{\circ}\text{C}$
Specific Gravity	0.86 – 0.87
Vapor Pressure, mmHg	< 2
Vapor density, Air=1	> 1
Volatiles, % by volume	< 2
Solubility in water	insoluble
Evaporation rate, butyl acetate=1	< 1

**10. Stability and Reactivity**

GENERAL

This product is stable and hazardous polymerization will not occur.

INCOMPATIBLE MATERIALS AND CONDITIONS TO AVOID

Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS

Combustion produces carbon monoxide, carbon dioxide along with thick smoke.

**11. Disposal Considerations**

WASTE DISPOSAL

Waste may be disposed of by a licensed waste disposal company. Contaminated absorbent material may be disposed of in approved landfill. Follow local, state and federal disposal regulations.

10/11/2007

CA# OC-07-0340

10 October 2007

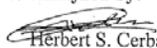
### Certificate of Analysis

Product : Fil-Ester 618  
 Description : Coco Methyl Ester  
 Quantity : 15 liters  
 Batch Number : 100807  
 Customer : Ryan Day  
 Center for Marine Studies  
 The Universities of  
 Queensland Brisland

We hereby certify that the above-mentioned product is manufactured and exported in good quality conditions as per our standard specification.

Properties	Results	Standard
Appearance	Clear Yellow Liquid	
Acid Value	0.33	0.50 max
Color, APHA	90 <sup>2</sup> -100	120 max
Moisture, %	0.08	0.10 max
Iodine Value	8.84	12 max

Analyzed by:

  
 Herbert S. Cerbas  
 Lab. Tech.

Certified by:

  
 Glenn C. Apostol  
 R&D Chemist II



Republic of the Philippines  
Department of Science and Technology

**INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE**  
*(Formerly National Institute of Science and Technology)*  
**STANDARDS AND TESTING DIVISION**  
Gen. Santos Ave., Bicutan, Taguig, Metro Manila 1631

Fax No.: (632) 837-31-67 / 837-00-32

Tel. Nos. 837-20-71 to 82  
local 2188, 2189

**TEST REPORT**  
No. 0906PTOX0107

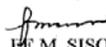
Customer's Name : Chemrez, Inc.  
Address : 65 Industria St. Bagumbayan, Q. C.  
Sample : Light yellow colored clear liquid in sealed plastic bottle  
Description : about 1 liter marked as *Fil Ester 618*  
Identification : Batch #: R 706352  
Date received : September 27, 2006  
Date (s) Tested : December 07 - 28, 2006

The median lethal dose (LD50) of the sample, administered orally to male ICR mice is  $73.0689 \pm 2.3465$  mL/kg. Toxidrome ranged from grooming, piloerection, straub tail, hyperemia, decreased motor activity, ptosis, ataxia, excretion of sample, increased motor activity, oily fur, alopecia and death of mice.

Details of Acute Oral Toxicity Test enclosed.

Test Method: Modified Acute Oral Toxicity in Mice; OECD, # 401, 1993

**VALIDITY OF THE REPORT:** The test results are those obtained at the time of the test and pertain only to the sample(s) received by this Laboratory. *Codes and words in italics* are quoted solely for the customer's reference; significance of these codes and words are not verified by this Laboratory. This report is not to be used for advertising purposes or sales promotion.

  
M. SISON  
Head, Pharmacology and  
Toxicology Laboratory

Issued under the Authority of:

  
HERMELINA H. BION  
Division Chief

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12/29/06 Page 1 of 4



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Department of Science and Technology

**INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE**  
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**STANDARDS AND TESTING DIVISION**  
Gen. Santos Ave., Bicutan, Taguig, Metro Manila 1631

Fax No.: (632) 837-31-67 / 837-00-32

Tel. Nos. 837-20-71 to 82  
local 2188, 2189

Sample : Light yellow colored clear liquid in sealed plastic bottle  
Description : about 1 liter marked as *Fil Ester 618*  
Identification : Batch #: R 706352  
Date received : September 27, 2006  
Date (s) tested : December 07 - 28, 2006

Data :

pH of sample : 5.6

Control: Distilled Water

Animals used: Male ICR mice (30-37 g)

Procedure:

Preliminary dosing was done to determine the expected dose that will cause 50% death of the experimental animals. Three (3) increasing log doses of the test substance were given orally to the animals in three (3) groups of 10. Another group of 10 animals was given the control, Distilled Water, equivalent to the volume used for the highest dose of the test substance. The number of deaths and other adverse abnormal signs and manifestations were closely observed and noted for the first two (2) hours after administration of the test sample. This was continued in the next 24 to 48 hours, daily up to 14 days.

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Tel. Nos. 837-20-71 to 82  
local 2188, 2189

Results:

Table 1. Behavioral Observation/Toxidrome after Oral Administration of Sample to Male ICR Mice

Dose mL/kg	No. of Mice	Observation
0 <sup>a</sup>	10	Twenty (20) minutes after dosing, the mice manifested decreased motor activity and grooming. All mice recovered within 24 hours. No other adverse/abnormal signs or death occurred within the 14 days period of observation.
60.00	10	Twenty (20) minutes after the first dosing, the mice manifested grooming, straub tail, piloerection, hyperemia, excretion of sample, decreased motor activity, ptosis followed by increased motor activity, ataxia, oily fur, alopecia and death of two (2) mice within 24 hours. The remaining eight (8) mice recovered after 24 hours. However, the alopecia of the remaining eight (8) mice was still observed up to 14 days period of observation.
77.46	10	Fifteen (15) minutes after the first dosing, the mice manifested grooming, straub tail, piloerection, hyperemia, excretion of sample, decreased motor activity, ptosis followed by increased motor activity, ataxia, oily fur, alopecia and death of three (3) mice within 24 hours. The remaining seven (7) mice recovered after 24 hours. However, the alopecia of the remaining seven (7) mice was still observed up to 14 days period of observation.
100	10	Twelve (12) minutes after the first dosing, the mice manifested grooming, straub tail, piloerection, hyperemia, decreased motor activity, ptosis, ataxia, excretion of sample followed by increased motor activity ataxia, oily fur and death of nine (9) mice within (24) hours; one (1) mouse died after two (2) days.

<sup>a</sup> control, the same volume as in the highest dose

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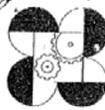


Table 2. Summary of Mortality Ratio of Mice Administered Orally with the Sample

Mortality Ratio =  $\frac{\text{Number of mice with positive sign (death)}}{\text{Total number of animals tested}}$

Group Number	Dose mL/kg	No. of Mice	Mortality Ratio				
			Day 1	Day 2	Day 3	Day 7	Day 14
I	0 a	10	0/10	0/10	0/10	0/10	0/10
II	60.0	10	2/10	2/10	2/10	2/10	2/10
III	77.46	10	3/10	3/10	3/10	3/10	3/10
IV	100	10	9/10	10/10	10/10	10/10	10/10

a- Control, the same volume as in highest dose

Animals, which died after drug administration and those sacrificed after fourteen (14) days had normal findings on the vital organs. Increased in weight were observed in all mice.

## 7.3 Cytosol

MATERIAL SAFETY DATA SHEET  
for  
**CytoSol Biosolvent**

EMERGENCY PHONE: 1-510-233-0102  
MSDS REFERENCE: **CytoCulture International, Inc.**

---

SECTION I - IDENTIFICATION

---

PRODUCT: **CytoSol Biosolvent** proprietary formulation includes methyl esters  
SYNONYMS: Formulation of methyl esters of plant (soy) oil and macronutrients  
CAS NO.: None  
SARA HAZARD: None noted (Section 311/312)  
Title III Section 313 - Not Listed  
DOT Regs: Not regulated by DOT.

---

SECTION II - INGREDIENTS AND HAZARD CLASSIFICATION

---

<u>COMPOSITION (Typical)</u>	<u>PEL/TLV</u>	<u>HAZARD</u>
METHYL ESTERS	NONE/NONE	NONE NOTED

PCBs: Not detected at or above reporting limit of 800µg/kg

<u>METALS ANALYSIS</u>	<u>RESULTS (mg/kg)</u>	<u>REPORTING LIMIT (mg/kg)</u>
ARSENIC	ND	2.4
CADMIUM	ND	0.24
CHROMIUM(Total)	ND	0.49
COPPER	ND	0.49
LEAD	ND	4.9
MERCURY	ND	0.091
NICKEL	ND	0.97
ZINC	ND	0.97

---

SECTION III - HEALTH INFORMATION

---

INHALATION: UNKNOWN - NONE SUSPECTED  
INGESTION: LD<sub>50</sub>>50 ML/KG (ALBINO RATS) (SIMILAR PRODUCTS)  
EYE CONTACT: SIMILAR PRODUCTS WERE NOT CLASSIFIED AS EYE IRRITANTS.  
SKIN CONTACT: METHYL SOYATE WAS NOT CLASSIFIED AS A PRIMARY SKIN IRRITANT OR CORROSIVE MATERIAL.

---

SECTION IV - OCCUPATIONAL EXPOSURE LIMITS

---

PEL: NO OSHA PEL  
TLV: NO ACGTH TLV

---

---

SECTION V - EMERGENCY FIRST AID PROCEDURES

---

FOR OVEREXPOSURE BY <u>SWALLOWING</u> :	Non-toxic, however, call a physician promptly.
FOR OVEREXPOSURE BY <u>SKIN CONTACT</u> :	Non-irritating, but wash affected area with soap & water.
FOR OVEREXPOSURE BY <u>EYE CONTACT</u> :	Immediately flush eyes with plenty of cool water for at least 15 minutes. Do not let victim rub eyes.
FOR OVEREXPOSURE BY <u>INHALATION</u> :	Non-irritating and non-toxic, but immediately remove victim to fresh air. If victim has stopped breathing, give artificial respiration, preferably by mouth-to-mouth. Get medical attention immediately.

---

SECTION VI - PHYSICAL DATA

---

BOILING POINT:	Over 400° F (204° C) at 760 MM Pressure
POUR POINT:	+10° F (-12° C) [ASTM D97-93]
VAPOR PRESSURE:	Less than 1 MM HG Pressure @ 162°F ( 72° C); NON EXPLOSIVE
SPECIFIC GRAVITY:	0.8877 @ 60°F (15.6°C) [ASTM D1298-85(90)]
SOLUBILITY IN WATER:	NEGLIGIBLE AT ROOM TEMPERATURE
APPEARANCE AND COLOR:	LIGHT AMBER TO CLEAR LIQUID AT ROOM TEMPERATURE
VISCOSITY:	4.15 CST @ 104°F (40°C) [ASTM, D92-90]

---

SECTION VII - FIRE AND EXPOSION HAZARDS

---

FLASH POINT/METHOD USED:	360° F (182° C) [ASTM, D445-88]
FLAMMABLE LIMITS IN AIR, % BY VOL. LOWER:	NOT APPLICABLE/NON-VOLATILE
FLAMMABLE LIMITS IN AIR, BY VOL. UPPER:	NOT APPLICABLE/NON-VOLATILE
NFPA RATING:	NO NFPA RATING
HMIS RATING:	<b>HEALTH (0) FIRE (1) REACTIVITY (0)</b>

SPECIAL FIRE FIGHTING PROCEDURES & PRECAUTIONS:

(INDIVIDUALS SHOULD PERFORM ONLY THOSE FIRE FIGHTING PROCEDURES FOR WHICH THEY HAVE BEEN TRAINED). USE WATER SPRAY, DRY CHEMICAL, FOAM OR CARBON DIOXIDE. WATER MAY BE INEFFECTIVE, BUT SHOULD BE USED TO KEEP FIRE-EXPOSED CONTAINERS COOL. WATER SPRAY MAY BE USED TO FLUSH SPILLS AWAY FROM FIRE.

USUAL FIRE & EXPLOSION HAZARDS:

OIL SOAKED RAGS CAN CAUSE SPONTANEOUS COMBUSTION IF NOT HANDLED PROPERLY. BEFORE DISPOSAL, WASH RAGS WITH SOAP AND WATER AND DRY IN WELL VENTILATED AREA.

FIRE FIGHTERS SHOULD WEAR SELF-CONTAINED BREATHING APPARATUS IN THE POSITIVE-PRESSURE MODE WITH A FULL FACEPIECE WHEN THERE IS A POSSIBILITY OF EXPOSURE TO SMOKE, FUMES OR HAZARDOUS DECOMPOSITION PRODUCTS (CO<sub>2</sub> AND CO).

---

---

SECTION VIII - REACTIVITY

---

STABILITY:	GENERALLY STABLE
HAZARDOUS POLYMERIZATION:	NONE LIKELY
CONDITIONS & MATERIALS TO AVOID:	AVOID CONTACT WITH STRONG OXIDIZING AGENTS, SOLVENT ACTION DISSOLVES RUBBER MATERIALS, STYROFOAM AND POLYURETHANE
HAZARDOUS DECOMPOSITION PRODUCTS:	COMBUSTION WOULD PRODUCE CARBON MONOXIDE AND CARBON DIOXIDE.

---

SECTION IX - EMPLOYEE PROTECTION

---

CONTROL MEASURES:	HANDLE IN THE PRESENCE OF ADEQUATE VENTILATION WHEN USED TO DISSOLVE CRUDE OIL AND SPILLED PETROLEUM.
RESPIRATORY PROTECTION:	RECOMMENDED EXPOSURE LIMITS (i.e. OSHA-PEL AND ACGH-TLV) HAVE NOT BEEN ESTABLISHED FOR THIS MATERIAL. WHETHER THERE IS A NEED FOR RESPIRATORY PROTECTION UNDER YOUR CONDITIONS OF HANDLING OF THIS MATERIAL SHOULD BE EVALUATED BY A QUALIFIED HEALTH SPECIALIST.
PROTECTIVE CLOTHING:	GLOVES RECOMMENDED FOR ANY APPLICATION INVOLVING THE CLEANUP OR DEGREASING OF WASTE OIL.
EYE PROTECTION:	WEAR SAFETY GLASSES PER ANSI STANDARD Z87.1

---

SECTION X - ENVIRONMENTAL PROTECTION

---

ENVIRONMENTAL PRECAUTIONS:	AVOID UNCONTROLLED RELEASES OF THIS MATERIAL. WHERE SPILLS ARE POSSIBLE, A COMPREHENSIVE SPILL RESPONSE PLAN SHOULD BE DEVELOPED AND IMPLEMENTED. PREVENT RELEASES TO WATER.
SPILL OR LEAK PRECAUTIONS:	CONTAIN SPILLED MATERIAL AND TRANSFER TO SECURE CONTAINERS. WHERE NECESSARY, COLLECT USING ABSORBENT MEDIA, WASH DOWN AREA WITH DETERGENT. IN THE EVENT OF AN UNCONTROLLED RELEASE OF THIS MATERIAL, THE USER SHOULD DETERMINE IF THE RELEASE IS REPORTABLE UNDER APPLICABLE LAWS AND REGULATIONS. USE DOUBLE CONTAINMENT IF POSSIBLE.
WASTE DISPOSAL:	ALL RECOVERED MATERIAL SHOULD BE PACKAGED, LABELED, TRANSPORTED, AND DISPOSED OR RECLAIMED IN CONFORMANCE WITH APPLICABLE LAWS AND REGULATIONS AND IN CONFORMANCE WITH GOOD ENGINEERING PRACTICES. AVOID LANDFILLING OF LIQUIDS. RECYCLE UNUSED PRODUCT AT REGULAR OIL COLLECTION FACILITY OR BLEND WITH BURNER FUEL.
MARINE TOXICITY:	<i>MYSIDOPSIS BAHIA</i> (U.S. EPA 1991) BIOASSAY: LC <sub>50</sub> = 122 PPM. <i>MENIDIA BERYLLINA</i> (U.S. EPA 1990) BIOASSAY: LC <sub>50</sub> = 578 PPM TOXICITY BELIEVED TO BE DUE TO RESTRICTED OXYGEN DIFFUSION ON WATER SURFACE BY FLOATING PRODUCT.
BIODEGRADABILITY:	BIODEGRADES IN SEAWATER WITH A HALF-LIFE OF 4 DAYS. IN EPA STANDARD BIODEGRADATION ASSAY, THE PRODUCT IS COMPLETELY BIODEGRADED TO CARBON DIOXIDE IN 28 DAYS

---

SECTION XI - REGULATORY CONTROLS

---

DEPARTMENT OF TRANSPORTATION:

**DOT CLASSIFICATION:** NOT REGULATED  
**DOT PROPER SHIPPING NAME:**  
**OTHER DOT INFORMATION:**

**OTHER REGULATORY REQUIREMENTS:** LISTED IN TSCA INVENTORY(SOY METHYL ESTERS)

**LISTED ON EPA'S NATIONAL CONTINGENCY PLAN (NCP)  
SCHEDULE OF PRODUCTS (1997) USED IN OIL SPILL CLEAN UPS**

**LICENSED IN CALIFORNIA AS A SHORELINE CLEANER FOR USE IN SENSITIVE AQUATIC  
OR MARINE ECOSYSTEMS DURING OIL SPILL CLEANUPS.**

---

SECTION XII-PRECAUTIONS: STORAGE, HANDLING AND USAGE

---

NO SPECIAL HEALTH PRECAUTIONS ARE NECESSARY. HOWEVER, CYTOSOL SHOULD BE HANDLED AND TREATED WITH THE SAME ENVIRONMENTAL SAFETY PRECAUTIONS AS ANY SOLVENT. AVOID CONTACT WITH STYROFOAM, POLYURETHANE AND NATURAL RUBBER PRODUCTS - SOLVENT ACTION WILL DISSOLVE THESE MATERIALS. DO NOT EXPOSE TO OLD PAINTED SURFACES, CAULKING, RUBBER OR OTHER POLYMERS NOT SPECIFICALLY DESIGNED TO HANDLE SOLVENTS. IN CASE OF SPILLS, WIPE UP OR VACUUM SPILLED PRODUCT AND IMMEDIATELY WASH CONTACT AREAS WITH DETERGENT AND WARM WATER. DO NOT ALLOW SPILL TO ENTER WATERWAYS OR STORM SEWERS.

The information presented herein is believed to be factual as it has been derived from the works and opinions of persons believed to have qualified experts; however, nothing contained in this information is to be taken as a warranty or representation for which CytoCulture International, Inc. bears legal responsibility. The user should review any recommendations in the specific context of the intended use to determine whether they are appropriate or contact CytoCulture for consultations.

PREPARED BY: Randall von Wedel, Ph.D.  
President & Director of Research

DATED: January 1997 Updated 2004

CytoCulture International, Inc.  
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## 8 Appendix D: Literature Cited

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## **9 Appendix E: Acknowledgements**

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