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# Effect of Bioremediation on the Microbial Community in Oiled Mangrove Sediments

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**Bioremediation was conducted in the field on a mature *Rhizophora stylosa* mangrove stand on land to be reclaimed near Fisherman's Landing Wharf, Gladstone Australia. Gippsland crude oil was added to six large plots (>40 $m^2$) and three plots were left untreated as controls. Bioremediation was used to treat three oiled plots and the remaining three were maintained as oiled only plots. The bioremediation strategy consisted of actively aerating the sediment and adding a slow-release fertilizer in order to promote oil biodegradation by indigenous micro-organisms. Oil addition stimulated the numbers of alkane-degrading bacteria slightly to levels of $10^4$–$10^5$/g sediment. Bioremediation of the oiled sediment had a marked effect on the alkane-degrading population, increasing the population size by three orders of magnitude from $10^5$ to $10^8$ cells/g of sediment. An effect of bioremediation on the growth of aromatic-degraders was detected with numbers of aromatic-degraders increasing from $10^4$ to $10^6$ cells/g of sediment. Active aeration and nutrient addition significantly stimulated the growth of hydrocarbon-degraders in oiled mangrove sediment in the field.** 

**Keywords: bacteria; bioremediation; degradation; enumeration; hydrocarbons; oil spills.**

A range of cleaning techniques have been successfully developed to limit the environmental impact caused by oil pollution. However, there is evidence that some of these techniques can be detrimental to the recovery of certain habitats (Sell, 1995). Emphasis has thus been steered towards techniques which assist the natural environmental processes to remove oil pollutants. Bioremediation is such a technique, and in the context of oil spills is aimed at stimulating the biodegradation rate of oil. This is achieved by supplying the indigenous hydrocarbon-degrading microbial community with electron acceptors and nutrients that are not present in sufficient amounts naturally. It is well known that petroleum-degrading bacteria occur extensively in a wide range of aquatic and marine environments, including sediment and the water column. Bioremediation has been proven to work on sandy, pebble and cobble shorelines both after experimental and real spill incidents (Prince, 1993; Bragg *et al*., 1994; Swannell *et al*., 1994, 1996, 1999b). However, little work has been done in mangroves (Burns *et al*., 1999). Mangrove sediments are predominantly anaerobic, comprising sulphidic muds (Alongi and Saeskumar, 1992). Generally, less than 1 cm of the surface sediment is aerobic. Preliminary studies on forced aeration for this study showed, that the oxygenation zone in a *Rhizophora* forest extended to a depth of ~1900 μm and thus, most of the area of mangroves plots was anoxic below 1–2 mm (Duke *et al*., 1999). Previous research suggests that in mangrove sediment the rate of hydrocarbon biodegradation by the bacterial community is likely to be limited by oxygen concentrations and nutrient levels (Swannell *et al*., 1994; Scherrer and Mille, 1989; Oudot and Dutrieux, 1989; Burns *et al*., 1999). Thus, in this study, a strategy of aeration and nutrient addition was trialed.

There are well-established methods for studying the size and composition of hydrocarbon-degrading microbial populations (e.g., Brown and Braddock, 1990; Wrenn and Venosa, 1996). The total culturable heterotrophic population can be estimated by assessing growth on an appropriate medium (e.g., Marine Broth 2216) which contains a range of nutrients and salts normally found in marine habitats. Bacteria capable of oil degradation can be enumerated based on their ability to

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grow selectively on particular hydrocarbon sources for example: those capable of growth on aromatic-hydrocarbons and those capable of growth on alkanes. Gippsland crude oil used in this study has a high concentration of aliphatic hydrocarbons (51% w/w) (Burns *et al*., 1999). Therefore we predicted that, if the bioremediation strategy was successful, a large increase in the number of alkane-degraders would occur. The aromatic content of the oil is considerably less (3% w/w) (Burns *et al*., 1999) than the alkane content. However, many aromatics are biodegradable (Prince, 1993) and are important from a toxicological perspective (Venosa *et al*., 1996). Therefore, we also monitored the influence of bioremediation on the numbers of aromatic-degrading micro-organisms.

Heterotrophic and hydrocarbon-degrading bacteria from untreated mangrove sediments and sediments to which oil was added were enumerated in the laboratory using a most-probable-number (MPN) method (Wrenn and Venosa, 1996). Although it is well established that it is generally unnecessary to add hydrocarbon-degrading micro-organisms as part of any bioremediation strategy for contaminated shorelines, we considered it to be important to estimate numbers of aromatic and aliphatic hydrocarbon-degraders in the mangrove sediments. The study was designed to provide fundamental information on the response of hydrocarbon-degrading bacteria in oiled mangroves and to evaluate bioremediation as an oil response strategy in mangrove forests of tropical coastal environments contaminated by oil.

## Materials and Methods

*Study site*

The Port of Gladstone is situated on the central coast of Queensland, Australia ~550 km north of Brisbane (23°47′25S, 151°10′1E) (Fig. 1). It is one of the State's major industrial centres and leading ports. The study site was located in a *Rhizophora stylosa* mangrove forest, on land to be reclaimed near Fisherman's Landing Wharf, the most westerly wharf within the Port of Gladstone.

*Experimental design and sediment sample collection*

The experiment used a randomized block design (Venosa *et al*., 1996). Each block consisted of an untreated control plot, a plot treated with Gippsland crude oil and a bioremediated plot treated with oil, aeration plus Osmocote™ fertilizer. Each plot was 6 m × 6 m long, contained between 13 and 28 mangrove trees and were between 25 and 150 m apart from one another at each site. The three blocks were located at the same height in the mid-intertidal range at Fisherman's Wharf. In Gladstone for the period of the study, the tide was diurnal with the mean high tide magnitudes varying from 3.9 m neap to 3.1 m spring tides and low tide magnitudes varying from 1.5 m neap to 0.7 m spring tides (Queensland Transport, 1997/1998). Osmocote™ fertilizer is a controlled release fertilizer consisting of 19% w/w nitrogen (9% nitrate-N; 10% ammonia-N), 2.5% w/w phosphorous (1.9% w/w water soluble), 10% w/w potassium (water soluble, chloride free), 4.8% w/w sulphur and 0.8% w/w calcium covered in an 8% w/w organic resin coating. Approximately 5.4 kg of Osmocote fertilizer was sprinkled evenly onto the sediment surface of each bioremediated plot at an application rate of ~0.15 kg $m^{-2}$.

Bioremediated plots were aerated using a device consisting of tubing and 39 aquarium airstones (each 10 cm long) which were buried 2–3 cm into the sediment around the roots of trees. The aeration system was supplied by three 12 V diaphragm air compressors, delivering more than 100 l per minute (Duke *et al*., 1999). Compressed air was piped in a common air supply line to the three bioremediated plots via 19 mm plastic irrigation tubing over distances up to 380 m from the common compressor station (Duke *et al*., 1999).

Aeration was supplied to the bioremediated plots for four months, between late August and mid December 1997. Fertilization of the plots was carried out 2 days and 6 months after addition of oil. Osmocote has been successfully used as a bioremediation agent to treat oiled shorelines elsewhere (Swannell *et al*., 1999a). Surface sediment samples of approximately 3 g were taken from within the 0–1 cm zone from four random regions within each plot and combined into one vial. Samples were transported back to the laboratory on ice. The wet sediment was homogenized by vortexing for 2–3 min and sub-samples were taken for further studies.

*Bacterial enumeration*

Hydrocarbon-degrading and heterotrophic bacteria were enumerated using a MPN method adapted from Wrenn and Venosa (1996). Bushnell Haas medium (composition: magnesium sulphate 0.2 g $l^{-1}$; calcium chloride 0.02 g $l^{-1}$; monopotassium phosphate 1 g $l^{-1}$; ammonium phosphate dibasic 1 g $l^{-1}$; potassium nitrate 1 g $l^{-1}$ and ferric chloride 0.05 g $l^{-1}$) supplemented with 2% (w/w) NaCl was used as the growth medium in 96 well microtitre plates. Specific hydrocarbon sources (hexadecane and a mixture of aromatic compounds, phenanthrene 0.5 g $l^{-1}$, anthracene 0.25 g $l^{-1}$, dibenzothiophene 0.25 g $l^{-1}$, carbozol 0.25 g $l^{-1}$ and fluorene 0.25 g $l^{-1}$) were added to stimulate the growth of alkane-degraders and aromatic-hydrocarbon degraders, respectively. The growth medium used for enumeration of culturable heterotrophic bacteria was Marine Broth 2216 (Difco).

Plates for enumeration of alkane-degraders were prepared by addition of 180 µl of Bushnell Haas medium per well, followed by 5 µl of filter sterilized hexadecane. For preparation of plates for enumeration of aromatic-degraders, a stock solution of the aromatic compounds was prepared in 250 ml of hexane. This solution (10 µl) was added to each well in the microtitre plates. Bushnell Haas medium (180 µl) was then added

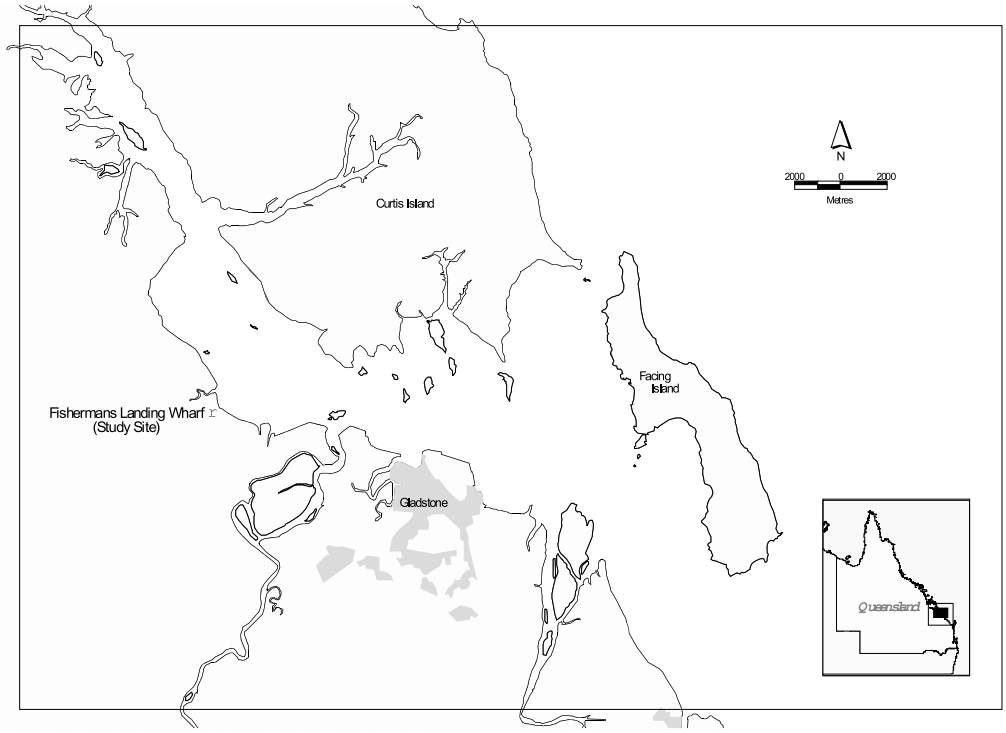


**Fig. 1** Study site near Fisherman's Landing Wharf, Gladstone, Qld, Australia.

to the wells. Marine Broth 2216 (180 µl per well) was used for enumeration of heterotrophic bacteria.

A sample of 3 g of sediment was placed in a vial containing 10 ml of Bushnell Haas medium supplemented with 2% (w/w) NaCl and mixed to form a slurry. One ml of this slurry was placed into a vial containing 9 ml of Bushnell Haas medium supplemented with 2% (w/w) NaCl. A dilution series was prepared from this sample, from $10^{-1}$ to $10^{-12}$, and used to inoculate microtitre plates. Eight wells were inoculated per dilution, with a 20 µl inoculum.

Plates for enumeration of aromatic-degrading bacteria were incubated at room temperature (26–27°C) for 6 weeks, whereas plates for enumeration of alkane degraders and heterotrophic bacteria were incubated for 2 weeks. After incubation, all plates were read using a Wiacalc microplate reader to determine positive or negative growth of bacteria. Alkane and heterotrophic plates were read using an 620 nm filter and aromatic plates were read using a 405 nm filter. Then 50 µl iodonitrotetrazolium violet (INT) solution (3 g $l^{-1}$) (Sigma) was added to each of the inoculated wells of the plates for alkane-degrading and heterotrophic bacteria. The plates were incubated with INT at room temperature overnight (26–27°C). Wells in the MPN plate for alkane-degrading and heterotrophic bacterial isolates were counted as positive if the INT was reduced to insoluble formazan, deposited intracellularly as a red precipitate (Wrenn and Venosa, 1996). Growth was assessed in wells of plates for enumeration of aromatic-degraders by monitoring the appearance of a yellow or brown colour (caused by an accumulation of partial oxidation products of aromatic substrates, Wrenn and Venosa, 1996). INT reduction and accumulation of partial oxidation products were monitored by absorbance readings, which increased in comparison to readings of sterile, control wells.

A most probable number computer program (version 4.04 by Klee, 1993) was used to estimate the number of alkane and aromatic hydrocarbon-degrading bacteria and heterotrophic bacteria. Analysis of variance (ANOVA) was used to determine if there were any significant differences in the counts of alkane-degrading, aromatic and heterotrophic bacteria in mangrove sediment and those treated with Gippsland oil and bioremediation at the three sites. Data were transformed ($\log_{10}$) and tested for normal distribution prior to statistical analysis. Multi-factorial ANOVA was computed on the data using the SYSTAT 6.0 for Windows analytical software program.

*Morphology and identification of bacteria*

A 1 ml sub-sample of the $10^{-1}$ dilution was taken from the MPN plates and plated onto marine agar, alkane agar or aromatic agar. The composition of the alkane and aromatic agar plates consisted of a basal Bushnell Haas medium supplemented with 2% (w/w) NaCl, agar, silica gel hydrocarbon powder with either hexadecane or a mixture of aromatic compounds (phenanthrene, anthracene, fluorene, dibenzothiophene, carbozol-concentrations as previously cited) and cycloheximide (75 mg/l) (Sigma) (adapted from Wunsche *et al.*, 1995). Silica gel hydrocarbon powder was made by adding 5 g of hydrocarbon compounds to 30 ml of

dichlromethane, mixing to form a paste and allowing to stand overnight. The powder was added at 10 g/l to the agar media. Cycloheximide is an antibiotic which restricts the growth of fungi and it was added to the media to inhibit fungal contamination. Phenol red dye was added (1% w/v) to alkane plates as it provided a good background to enumerate bacteria. Marine agar and alkane plates were incubated for a period of two weeks and aromatic plates were incubated for a period of 6 weeks, all at 27°C. After incubation, mixed colonies of bacterial isolates were purified and their colony morphology observed, at 10 × magnification. All bacteria were stored by cryopreservation at −80°C.

The predominant 22 isolates were selected out of a collection of 150 morphologically distinct strains for identification using a range of primary and secondary characterization tests, in order to classify them to a genus level. Primary tests included gram stain, colony morphology, catalase and oxidase, oxidation/fermentation and motility. Secondary tests included growth on specific media (MacConkey, Dnase and Marine Agar), susceptibility to 0/129 and penicillin, utilization of hydrocarbons as a sole carbon and energy source, starch hydrolysis, requirement of sodium ions, citrate utilization test, Tween 80 hydrolysis, fermentation and gas production from glucose and fermentation from D-mannitol. Some isolates presumptively identified as members of the Enterobacteriaceae, were further identified using the API 20 E identification system (BioMerieux, Marcy-l'Etoile, France) for confirmation to the genus level. Isolates were grown on Marine Agar to obtain pure cultures. Single colonies were suspended in sterile 3% (w/w) NaCl solution to obtain a turbidity equivalent to that of the MacFarland standard of 0.05. This solution was used as the inoculum in the API strips. API strips were prepared according to the API 20 E instruction manual version C. The API strips were incubated at 30°C for 24 h and results were recorded and analysed using Apilab Plus Software.

*Degradation of hydrocarbons by bacterial isolates*

Six of the 22 predominant isolates belonging to the genera *Serratia, Alicaligenes*, *Micrococcus*, *Aeromonas*, *Vibrio* and *Pseudomonas* were tested for the ability to degrade hydrocarbons. Stock solutions of hydrocarbons (alkanes: C17, C22, C24; aromatics: anthracene, dibenzothiophene, fluorene, carbazole) were prepared at a concentration of 1 mg $ml^{-1}$ of each component in dichloromethane. Stock solutions (200 µl) were added to flasks and the dichloromethane allowed to evaporate. Bushnell Haas medium (100 ml) supplemented with 2% NaCl was added to flasks. Inocula grown on Marine Agar 2216 were each inoculated into two separate sets of triplicate flasks. Uninoculated sets of flasks were maintained as controls. Flasks were incubated at 27°C for two and three weeks for alkanes and aromatics, respectively.

Contents of flasks were extracted with 30 ml hexane. Extraction efficiency was monitored by addition of 100 µl of a 1 mg $ml^{-1}$ in 1 solution of *o*-terphenyl to each flask. Extracts were reduced in volume by rotary evaporation, water was removed by adsorbtion with $Na_2SO_4$ and extracts were further evaporated under a nitrogen stream to a volume of 900 µl. An internal standard (1-*n*-eicosene, C20:1) was added to a final concentration of 1 mg $ml^{-1}$ in 1 ml samples. Samples were analysed with a Fisons GC 8000 gas chromatograph with a flame ionization detector (GC–FID). The column was 30 m DB-5 fused silica (J&W Scientific).

## Results

Counts of aerobic, heterotrophic bacteria in sediment samples from control plots remained relatively constant during the study period, ranging from $10^5$ to $10^6$ cells $g^{-1}$ of sediment (Fig. 2(a)). In oiled and bioremediated plots, there was approximately a 100-fold increase in the heterotrophic bacterial counts during the period of aeration, compared with counts in samples from control plots. This increase was statistically significant ($p < 0.05$). After day 60, there was a slight increase in the numbers of heterotrophic bacteria in samples from bioremediated plots compared with oiled and unfertilized plots (Fig. 2(a)). After day 120, the numbers of heterotrophic bacteria in bioremediated and oiled plots declined slightly, from $10^8$ to $10^7$ cells $g^{-1}$ of sediment, but remained higher than counts in control sites (Fig. 2(a)).

The numbers of alkane-degrading bacteria in control plots remained relatively constant at $10^4$. Numbers of alkane-degraders increased initially after oil and fertilizer addition (Fig. 2(b)). Numbers of alkane-degrading bacteria were significantly higher ($p < 0.05$) in bioremediated plots compared to oiled and control plots through most of the study period. Increases in the numbers of alkane-degrading bacteria coincided with the period of aeration. After day 120, the numbers of alkane-degraders decreased at all sites. Analysis of variance indicated significant differences ($p < 0.05$) in counts of alkane-degrading bacteria between control, oiled and bioremediated plots.

Counts of aromatic-degraders in control plots showed no significant change and remained relatively constant at $10^3$ cells $g^{-1}$ of sediment (Fig. 2(c)). However, the numbers of aromatic-degraders in oiled and bioremediated plots increased from $10^3$ to between $10^4$ to $10^6$ cells $g^{-1}$ of sediment over a period of 120 days. Generally, the numbers of aromatic-degraders in the oil-treated plots and bioremediated plots were lower than numbers of aromatic-degraders in corresponding plots (Fig. 2(b) and (c)). Between day 7 to day 90 (i.e., during the aeration period), there was a 10-fold increase in counts of aromatic-degraders in oiled and bioremediated plots, compared with counts in control plots (Fig. 2(c)). From day 90 to day 120, there was an additional 10–100-fold increase in counts of aromatic-

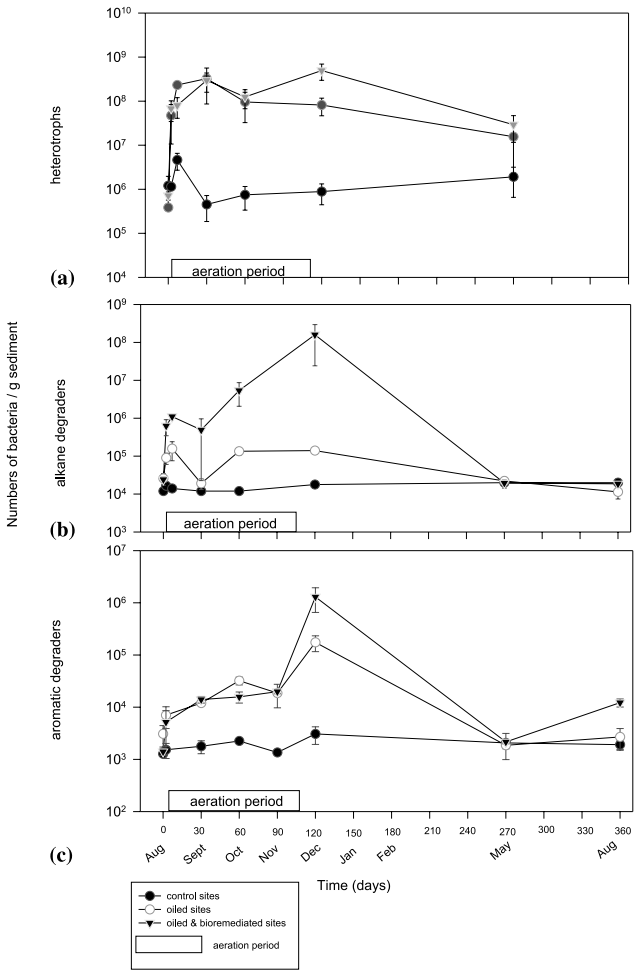


**Fig. 2** Bacterial counts in sediment samples from Gladstone experimental sites: (a) total heterotrophic bacterial counts, (b) counts of alkane-degrading bacteria, (c) counts of aromatic-degrading bacteria. Counts are averages of three samples and SE are indicated. The period of aeration is marked by the bar on the *x*-axis.

degraders in bioremediated and oiled plots (Fig. 2c). After day 120, the numbers of aromatic-degraders generally decreased, from $10^6$ to between $10^3$ and $10^4$ cells $g^{-1}$ of sediment.

In summary, there was a statistically significant effect of treatment type (bioremediation and oiling alone) on numbers of total heterotrophic, alkane-degrading and aromatic-degrading bacteria ($p < 0.05$) (see Table 1).

Over 150 strains of putative hydrocarbon-degrading bacteria were isolated from mangrove sediment at the study sites. Twenty-two bacterial isolates were studied in sufficient detail to assign them to genus level. These isolates were assigned the genera *Aeromonas* (1 strain), *Alcaligenes* (6 strains), *Alteromonas* (1 strain), *Micrococcus* (1 strain), *Moraxella* (2 strains), *Pseudomonas* (2 strains), *Photobacterium* (1 strain), *Serratia* (2 strains), *Shewanella* (1 strain) and *Vibrio* (5 strains).

The six microbial isolates tested for ability to degrade hydrocarbons were all capable of degrading both alkanes and aromatic hydrocarbons. *Serratia*, *Alicaligenes*, *Aeromonas*, *Vibrio* and *Pseudomonas* strains degraded the three alkane compounds at rates of between 11.7 and 14.2 μg $d^{-1}$. Degradation rates for aromatic hydrocarbons were slower and ranged between 6.1 and 8.0 μg $d^{-1}$ for fluorene, 3.2 and 8.0 μg $d^{-1}$ for dibenzothiopene, 1.6 and 2.8 μg $d^{-1}$ for anthracene and 1.4 and 4.7 μg $d^{-1}$ for carbazole. The Gram-positive *Micrococcus* strain degraded alkanes at a slower rate than the five Gram-negative strains (7.8 and 7.9 μg $d^{-1}$ for C22 and C24, respectively) but degraded aromatic compounds at comparable rates (8.0, 8.0, 2.3 and 2.4 μg $d^{-1}$ for fluorene, dibenzothiopene, anthracene, and carbazole, respectively).

## Discussion

Numbers of aromatic- and alkane-degrading bacteria increased markedly in oiled sites, both with and without bioremediation, compared with control sites. The most dramatic increase was in the numbers of alkane-degrading bacteriain oiled sites receiving bioremediation treatment. By the end of the aeration period, there were 1000 times more alkane degraders in the bioremediated plots compared with oiled plots that did not receive bioremediation treatment. This increase is greater than any previously reported. In previous field research, increases in the numbers of hydrocarbon-degraders that have been observed as a result of bioremediation treatments, have generally been approximately 10-fold (Prince *et al*., 1993; Swannell *et al*., 1999b), although

**TABLE 1**
Summary of ANOVA statistics data.[a]

| Source | Sum of squares | d.f. | Mean-square | *F*-ratio | *p* |
|---|---|---|---|---|---|
| Treatment | 35.029 | 2 | 17.515 | 49.657 | 0.000 |
| Bacterial type | 295.72 | 2 | 147.86 | 419.21 | 0.000 |
| Time | 41.39 | 6 | 6.9 | 19.56 | 0.000 |
| Bacterial Type × Treatment | 20.628 | 4 | 5.157 | 14.62 | 0.000 |
| Time × Treatment | 16.083 | 12 | 1.34 | 3.8 | 0.000 |
| Time × Bacterial Type | 23.01 | 12 | 1.918 | 5.437 | 0.000 |
| Time × Bacterial Type × Treatment | 15.704 | 24 | 0.654 | 1.855 | 0.015 |
| Error | 44.441 | 126 | 0.353 | | |

[a] Note that *p*-value = 0.05.

many authors have detected no effects on microbial numbers despite there being evidence that the oil was more rapidly biodegraded on bioremediation plots (Swannell *et al*., 1996; Venosa *et al*., 1996). The results in this study support the view that mangrove sediments are nutrient limited and nutrient addition can increase numbers of alkane-degrading bacteria. After day 120, the numbers of alkane-degraders decreased, a finding which may reflect the depletion of the readily degradable alkanes. However, even after 12 months, sediments contained measurable alkane concentrations (Burns *et al*., 2000). It is therefore more likely that the decrease in numbers of alkane-degraders after 120 days, shortly after cessation of aeration of sediments, is caused by oxygen limitation.

There was a lag time of approximately one to two months before large increases in numbers of both alkane- and aromatic-degrading bacteria (Fig. 2). Chemical analysis of total hydrocarbons and individual alkanes from Gippsland crude oil in the sediment samples (at 40 h, and 1 and 2 months) showed no evidence of change in the composition of hydrocarbons during this period (Duke *et al*., 1999). It is likely that oil was lost predominately to tidal washing since no degradation products were evident during this initial period. Over this two-month period, 90% of total hydrocarbons were lost from sediments by evaporation and dissolution (Duke *et al*., 1999). Therefore, only 10% of the initial hydrocarbons remained for degradation by the microbial community. Degradation of hydrocarbons was evident in samples taken after two months (Duke *et al*., 1999), presumably as a result of microbial action. A similar delay in biodegradation of one to three months was observed by Oudot and Dutrieux (1989) in oiled mangrove sediments in Kalimantan.

Numbers of alkane-degraders in both oil treated and bioremediated plots were generally higher than numbers of aromatic-degraders. Gippsland crude oil possesses a high proportion of alkanes in relation to the total hydrocarbon content (Burns *et al*., 1999). Also, alkanes have less complex chemical structures which may be easier to degrade (Atlas, 1981; Leahy and Colwell, 1990). Both these factors are likely explanations for the high counts of alkane-degraders.

Chemical analysis of hydrocarbons present in the sediments, sediments from oiled and bioremediated plots still contained measurable alkane concentrations after 12 months (Burns *et al*., 2000). This indicates that residual oils had weathered to only stage 3 to 4 in the petroleum geochemical degradation scale summarized by Volkman *et al*. (1984) and Peters and Moldowan (1993). It is possible that the numbers of aromatic-degraders will increase substantially only when the concentration of the more readily degradable alkane hydrocarbons is very low, as found in other cases (Volkman *et al*., 1984; Rowland *et al*., 1988). At day 270, there was some indication of an increase in numbers of aromatic-degraders in bioremediated plots (Fig. 2(c)), supporting this possibility. Alternatively, the lower numbers of aromatic-degraders may simply reflect the relatively low amounts of aromatics in Gippsland crude in comparison to the alkanes.

The numbers of alkane- and aromatic-degrading bacteria present in mangrove sediments indicate that the indigenous community has a considerable potential to degrade oil components. Previous studies in a variety of intertidal habitats have determined that the degradation of hydrocarbons by micro-organisms is mediated by a wide range of bacterial genera. The 22 presumptive hydrocarbon-degrading isolates identified to the genus level in this study fell into 10 genera, including *Pseudomonas*, *Vibrio*, *Aeromonas*, *Alcaligenes*, and *Micrococcus*, all genera previously reported to include common hydrocarbon-degrading bacteria (Cundell and Traxler, 1973; Walker and Colwell, 1974; Hamilton, 1990; Leahy and Colwell, 1990). Six of these isolates, from the genera *Serratia*, *Alicaligenes*, *Micrococcus*, *Aeromonas*, *Vibrio* and *Pseudomonas*, were directly shown to be capable of hydrocarbon degradation. In general, alkanes were degraded more rapidly than aromatic hydrocarbons, as is generally found with hydrocarbon-degrading bacteria (Leahy and Colwell, 1990).

Hydrocarbons occur naturally in the environment, and it is not surprising that micro-organisms have evolved the ability to utilise these compounds (Atlas, 1981). Additionally, mangrove forests are very organic rich environments, hence the number of different hydrocarbon-degrading microbial isolates is not surprising. Our results suggest that bioremediation based on aeration and nutrient addition substantially promotes the growth of alkane-degrading bacteria in tropical mangrove sediments and may therefore be a useful strategy to accelerate degradation of oil contamination in this environment.

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