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Microbial and Environmental Implications for Use of Monolayers to Reduce Evaporative Loss from Water Storages

**Pam Pittaway and
Tania van den Ancker**

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Microbial and Environmental Implications for Use of Monolayers to Reduce Evaporative Loss from Water Storages

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Executive Summary

While the concept of applying surface films to water bodies to reduce evaporative loss is an old one (La Mer, 1962), in practice, the economic and environmental costs of applying an artificial film thick enough to reduce evaporation has been limited to laboratory studies. The exception is with monolayers.

A monolayer occurs when the organic compound applied contains a hydrophilic polar anchor and a hydrophobic tail, with adjacent tails packing together to form a condensed surface film only one molecule thick (Barnes & Gentle, 2005). The most commonly studied artificial monolayer compound is based on hexadecanol, a saturated fatty alcohol with a chain length of 16 carbon atoms. The results of large scale trials using formulations based on hexadecanol have been variable (Frenkiel, 1965; Barnes, 2008), limiting the commercial adoption of the technology. The variable performance of hexadecanol has been attributed to bacterial decomposition, vaporisation and impurities on the water surface disrupting the physical packing of the monolayer (Barnes, 2008).

In this project, the properties of surface and subsurface water samples taken from water storages were examined to assess how water quality might influence the effectiveness of artificial monolayer compounds in reducing evaporative loss. The samples were collected from six water bodies in Southeast Queensland based on:

- The type of riparian vegetation in the immediate catchment (cleared agricultural land or woody remnant vegetation); and
- The main source of the water (overland flow, bore water or pumped from a watercourse during peak flow).

Water quality was assessed as follows:

- Biochemical oxygen demand (BOD) and the permanganate index were used to characterise the microbial and biochemical reactivity of the water;
- Absorbance in the ultraviolet (UV) region was used to indicate the concentration of humus-like organic compounds in the water samples; and
- Laboratory bioassays were developed to compare the resilience of several monolayer compounds to microbial degradation. The monolayer compounds selected were hexadecanol, octadecanol (a saturated fatty alcohol with 18 carbon atoms in the chain) and monoM (an experimental compound).

Water samples were also collected from water troughs filled with potable water that had been coated with three artificial surface films (two monolayer compounds and one silicone film) to examine how these products affected the quality of surface and subsurface water samples.

The results of the water quality survey indicate that all six samples have a natural surface film (a microlayer). Water quality attributes recorded for the six water bodies are at the high end of the median values recorded for surface water in the Condamine-Balonne-Culgoa catchment (Kenway, 1993). The hydrophobic organic materials responsible for forming microlayers include long-chain acids and hydrocarbons (Meyers & Kawka, 1982) derived from the waxes and phenolic compounds present in leaf litter and the bark of plants.

The properties of the microlayer samples were compared by calculating the enrichment factor (the value of a given parameter recorded for the microlayer water sample divided by the corresponding value of the subsurface water sample, (Estep et al., 1985). For the water bodies studied in this report, enrichment was greatest for the permanganate COD, BOD and absorbance in the UV spectrum. Very little difference was observed for pH, electrical conductivity and chloride concentrations. The enrichment values for UV absorbance for the Southeast Queensland water bodies are comparable to humic lakes studied in Northern Europe (Kostrzewska-Szlakowska, 2005). Results for the Southeast Queensland water bodies for the permanganate COD are mostly higher than values recorded for Norwegian humic lakes (Hessen, 1985).

The high incidence of humified compounds in the microlayer of Australian water bodies may increase the rates of microbial photochemical degradation of artificial monolayer compounds applied to water storages. The similarity of the physical and chemical properties of microlayer and monolayer compounds indicates that microlayer compounds may act as impurities, disrupting the spatial arrangement and stability of artificial monolayers. The main source of humified organic compounds in the microlayer of lakes in Poland is sphagnum peat (Hillbricht-Ilkowska & Kostrzewska-Szlakowska, 2004).

The high content of waxes and lignin in bark and leaves shed by Australian vegetation and litter fall coinciding with seasonally dry periods (Bunn, 1986) may explain why microlayer enrichment in this study is as high or higher than values recorded for European humic lakes. Water quality results suggest that new monolayer compounds

may need to be developed to match the range of microbial and photochemical activity and water quality attributes of Australian water storages.

In this study, the microbial resilience of the three monolayer compounds was compared using bacterial species isolated from activated sludge. The bacterial isolates recovered (*Acinetobacter* and *Pseudomonas*) are common in freshwater ecosystems and have been used as indicators of water quality (Lemke et al., 1997). Inoculated broth cultures using a monolayer compound as the sole organic carbon substrate were destructively sampled to monitor changes in monolayer concentration and bacterial population growth over the four-day incubation period of the assay. A gas spectrometer was used to quantify the residual concentration of monolayer compound. A nanospectrometer was used to quantify genomic DNA extracted from the broth. All three monolayer compounds were used by the microbes to some extent during the assay. Hexadecanol was the most readily degraded compound, with the concentration of bacterial DNA increasing steadily over the four days. The microbial use of octadecanol was slower, with monoM being used the least.

These results suggest that the microbial resilience of the monolayer can be improved by increasing the chain length by as little as two carbons (the difference between hexadecanol and octadecanol). Historically, all three compounds have been tested for how effective they are in reducing evaporative loss (Barnes, 2008). Unfortunately, the most microbially labile of the compounds, hexadecanol, has been the one most commonly applied in field trials. This poor performance of hexadecanol explains why commercial uptake of the technology has been limited. By increasing the carbon chain length or by using a different chemical structure (monoM), artificial monolayers applied to water storages will last longer and be more effective, improving the commercial feasibility of the technology.

The fact that all of the water storages studied have natural microlayers suggests that the artificial monolayers may not have as big an impact on fresh water ecology as first believed. Natural microlayer thickness varies from 100 to 500 nm (Munster et al., 1998). The thickness of artificial monolayers is about 1 to 10 nm (Norkrans, 1980). Microlayers reduce oxygen transfer by damping capillary waves and, by increasing surface temperature, increase the thickness of the laminar layer (Gladyshev, 2002). Monolayers also increase the thickness of the laminar layer and will affect oxygen transfer. Provided the change induced by applying a monolayer stays within the range recorded for natural microlayers, the adverse impact of artificial monolayers on freshwater ecology should be minimal. However, compounds other than monolayers,

applied either as the active ingredient or as part of the formulation, can adversely affect water bodies.

In this study, the impact of a silicone oil film and two monolayer films (hexadecanol and monoM) on water quality was compared. All three films reduced the surface tension of the water when sampled immediately after application. The hexadecanol-hydrated lime formulation increased the pH of the microlayer water sample from 8.2 to 10.9 units. The elevated pH reduced over time, and did not appear to affect the BOD. The BOD for the hexadecanol-hydrated lime treatment was five to six times higher than for the clean water sample, and 60 per cent higher than the monoM formulation, which did not contain hydrated lime. The BOD results for the silicone surface film suggest that the oil is an oxygen scavenger. When the oil is applied to a water surface and open to the air, this ability to scavenge oxygen may not be detrimental, however, these results highlight the importance of comparing the effect of surface water treatments on both surface (microlayer) and subsurface water samples. The impact of artificial films applied to water bodies will be greatest on the dynamics of the microlayer. This must be considered in designing any investigation of the impact of artificial surface films on water quality and aquatic ecology.

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1. Introduction

1.1. Monolayers as a Strategy for Reducing Evaporative Loss

1.1.1. Artificial Monolayers and Slowing Evaporative Water Loss

Some organic liquids are hydrophobic and form a film on the surface of water (Barnes & Gentle, 2005). While applying surface films to water bodies to reduce evaporative loss is an old concept (La Mer, 1962), in practice, the economic and environmental costs of applying artificial films thick enough to reduce evaporation has limited their application to laboratory studies. The exception is with monolayers. A monolayer occurs when the organic compound applied contains a hydrophilic polar anchor and a hydrophobic tail, with adjacent tails packing together to form a condensed surface film only one molecule thick (Barnes & Gentle, 2005).

Oxygen in alcohols is an example, which, in combination with non-polar, hydrophobic hydrocarbons can form compounds termed amphiphiles (Greek for loving both, in this case, water and oil). In contrast to the more miscible short-chain amphiphiles such as methanol, ethanol and propanol, longer chain alcohols spread to form an insoluble monolayer over the water surface.

The long-chain, linear, saturated alcohols greatly reduce evaporative loss, a property that is correlated with an increase in specific resistance as the monolayer is laterally compressed. The long-chain molecules are parallel and closely packed in a condensed monolayer. The head groups interact with water molecules and each other, and the hydrocarbon chains interact with nearby hydrocarbon chains (Barnes & La Mer, 1962). However, if impurities such as non-homologous amphiphilic hydrocarbons are added, for example cholesterol, the evaporative resistance of the monolayer may reduce substantially. The impurities occupy the spaces between the ordered clusters of monolayer molecules producing large holes. The efficiency of the monolayer in slowing evaporation can be reduced by 90 to 99 per cent if as little as one per cent of the area of the monolayer is occupied by holes.

To slow evaporative loss, most of the surface area of the water must be covered or the monolayer must be compressed by wind or wave action (Barnes & La Mer, 1962). Spontaneous spreading is essential for initial application and for 'self-repair' after turbulence or other disturbances to the monolayer. Chain lengths of C₁₈ and above are better at slowing evaporative loss, but they spread more slowly than molecules of shorter chain length. A mixture of a short with a long-chain saturated fatty alcohol

(homologous amphiphiles) can to achieve spreading and maintain high evaporation resistance. Fatty alcohols with chain lengths of C₁₆ and less will spread spontaneously and will readily mix with longer chain compounds. Under pressure, the longer chain amphiphiles squeeze out the shorter chain molecules, re-establishing the ordered, tightly-packed monolayer structure required to reduce evaporative loss. The shorter chain compounds may be lost to the system as a result of evaporation from the surface or dissolution in the subsurface water below the monolayer.

1.1.2. Biological Factors Affecting the Performance of Artificial Monolayers

Because they spread spontaneously and have high evaporative resistance, the C₁₆ and C₁₈ saturated fatty alcohols hexadecanol and octadecanol have been the most commonly studied monolayer compounds. Monolayers of hexadecanol can reduce evaporative loss from reservoirs by up to 50 per cent (Barnes, 1993). To be commercially feasible, the monolayer needs to stay on the water surface for four to five days (Chang et al., 1962). However, the bacterial species *Pseudomonas* and *Flavobacterium* can use hexadecanol as a carbon source, growing enough in two days to irreversibly damage a monolayer applied to a pure water surface. When the hexadecanol was applied to a solution containing an established bacterial population, the efficacy of the monolayer was lost within one or two hours. Both genera of bacteria are common in aquatic systems. *Burkholderia* (formerly *Pseudomonas*) *cepacia* and *P. putida* use a diversity of substrates from simple sugars such as glucose to complex hydrocarbon pollutants such as phenoxy acetic acids (Lemke et al., 1997). Together with a third species, *Acinetobacter calcoaceticus*, these bacteria have been used to indicate the microbial quality of polluted streams (Lemke & Leff, 1999).

Water quality will have a large effect on both chemical and biological conditions at the water surface. Processes such as photodegradation are likely to be greater in eutrophic water, where the concentration of humified and particulate organic carbon is higher. Many organic hydrocarbons undergo photochemical transformations when exposed to sunlight (Kawaguchi 1992). Humic-like organic compounds are known to promote the photodegradation of the organic compound triazine Irgarol 1051 (Amine-Khodja et al., 2006), and the rate of photolysis of the ester 2-chlorophenol increased as the permanganate COD of the water increased (Kawaguchi, 1992). Potassium permanganate oxidises particulate and humified organic carbon (Mrkyra, 1983; Tirol-Padre & Ladha, 2004), as well as microbial cells (Peterson et al., 1995). Oxidation of water samples with potassium permanganate can be used to quantify the concentration of humified compounds in water samples.

1.2. Surface Films on Natural Water Bodies

1.2.1. Microlayers in Freshwater Ecology

Early quantitative studies indicated that natural films commonly occurring on water bodies such as lakes and ponds induce surface pressures exceeding 30 dynes cm^{-1} (Goldacre, 1940). Later studies indicated that the thickness of these natural films varies from 100 to 500 μm (Munster et al., 1998). Natural surface films reduce oxygen transfer by damping capillary waves and by increasing the surface temperature, resulting in a thicker laminar layer (Gladyshev, 2002).

Studies on the biological consequences of naturally occurring surface films have focused mainly on the dynamics of microbial populations. Heterotrophic bacteria and fungi may be affected by and interact with the molecules comprising the surface film, changing their properties (Marshall, 1976). The thin layer of water and associated organic film bounded on the upper surface by the air interface and on the lower surface by the water interface is referred to by biologists as a microlayer. Microbes occupying the microlayer habitat are referred to as neuston, as distinct from the plankton occupying the bulk subsurface water (Gladyshev, 2002). Bacteria, fungi, total particulate matter and the nutrients nitrogen, phosphorus and trace elements are much more concentrated in the microlayer of freshwater ecosystems than in the bulk subsurface water (Hatcher & Parker, 1974; Munster et al., 1998). The hydrophobic organic materials responsible for forming microlayers include long-chain acids and hydrocarbons (Meyers & Kawka, 1982).

1.2.2. Sources of Natural Organic Inputs for Microlayers

Most studies on the composition of aquatic microlayers have focused on marine systems. Marine microlayers contribute significantly to the substrates available for microbial growth, with anthropogenic inputs from oil spills and pesticide use interacting with the function and biology of these surface films (Hatcher & Parker, 1974). In seawater, unbranched C_{12} to C_{18} fatty acids and alcohols are produced by algae, whereas C_{22} to C_{28} compounds are derived from land plants. Phytoplankton contribute to the C_{17} n-alkane concentration and bacteria synthesise branched lipids, and fatty acids and hydrocarbons with anteiso branching. The land-derived fatty acids and hydrocarbons are transported to the sea as adsorbed wax films coating eolian dust particles.

In Australia, leaf litter from trees breaks down to produce particulate organic matter with a high concentration of hydrophobic waxes (Franco et al., 1995). Under low rainfall

and nutrient conditions that limit microbial activity, these waxes become adsorbed onto sand particles. If enough sand grains become coated, the physical interaction between the grains can make the soil hydrophobic. Waxes associated with hydrophobic sands include polar and non-polar wax extracts, such as straight- and branched-chain fatty acids and fatty esters, alkanes, phytols, phytanols and sterols (Franco et al., 2000, Table 1).

The higher levels of lignin and phenolic compounds naturally present in Australian native vegetation can colour the surface water, reducing the growth potential for photosynthetic aquatic microbes (Song Qiu et al., 2005). These compounds become concentrated in water bodies as a result of leaf drop coinciding with a decrease in rainfall. In contrast, leaf fall in Europe coincides with higher rainfall, suggesting that changes in the seasonal development of microlayers in Australian water bodies may be greater than that in European and North American lakes (Bunn, 1986). To date the only scientific studies on microlayers in Australia have been on marine ecosystems (Marshall, 1976).

Studies in less arid, temperate forest lakes in Europe indicate that microlayers forming on the surface of small lakes contain much higher concentrations of total and organic nutrients than in the subsurface (Munster et al., 1998). Microbial biomass was a factor of eight to 280 times higher in the microlayer than in the subsurface water, and microbial activity (measured as radioactively-labelled glucose uptake) was four to five times higher in the neuston community relative to the plankton community. The authors concluded that the organic substances comprising the microlayer provide critical substrates for neuston microbes, supporting a foodweb of primary producers, decomposers, and micrograzers.

In the relatively small lakes studied (catchment area ranged from 4 to 30 ha, maximum depth ranged from 4 to 8 m respectively), the microlayer habitat provides better growing conditions for heterotrophic microbes than the subsurface water. In larger lakes with potentially larger mixing depths, wave action and the circulation of nutrients from the sediments may contribute more to the productivity of the system than the microlayer derived from land-based plant material.

The concentration of dissolved organic matter in marine and freshwater microlayers ranges from 1 to 5 mg/L and from 1 to 50 mg/L respectively (Norkrans, 1980). Lipids are the major constituents of microlayers, with amphiphilic molecules packing together to form an ordered film in the same manner as monolayers (refer to Section 1.1.1).

Saturated and unsaturated even-numbered chain lengths of 12 to 22 carbon atoms occur most often, with palmitic acid (C16: 0, '16' referring to the carbon number and '0' to the number of double bonds in the molecule) the most common. Short-chain fatty acids and alcohols are more concentrated in the immediate subsurface water than in the microlayer, suggesting that they have been forced out as a result of competitive adsorptive processes (longer chain molecules are less water soluble, more surface-active).

Table 1. Candidate amphiphilic hydrocarbons slowing evaporative loss.

Artificial monolayer compound	Carbon chain length and Common names	Examples of natural water repellent sand wax compounds
Dodecanol Dodecanoic acid	C ₁₂ Lauryl alcohol Lauric acid	Dodecanoic acid,
Tetradecanol Tetradecanoic acid	C ₁₄ Myristyl alcohol Myristic acid	Tetradecanoic acid, tetradecanoic acid methylester, tetradecanoic acid 12-methyl
Hexadecanol Hexadecanoxy ethanol Hexadecoxy ethanol Hexadecanoic acid	C ₁₆ , Cetyl alcohol Palmitic acid	Hexadecanoic acid, hexadecanoic acid methylethylester
Octadecanoic acid Octadecanol Octadecanoxy ethanol Ethyl octadecanoate Calcium octadecanoate	C ₁₈ , Stearic acid Stearyl alcohol	Octanoic acid, octadecadienoic acid methylester, octadecanoic acid, octadecanamide, oleic acid
Calcium nonadecanoate	C ₁₉	
Eicosanoic acid Calcium eicosanoate Eicosanol Octadecoxy ethanol Vinyl octadecanoate	C ₂₀ , Arachidic acid Arachidic alcohol Vinyl stearate	Eicosane, eicosane cyclohexane, eicosanoic acid methyl ester, eicosylester, eicosadienoic acid methyl ester
Octadecyl acrylate	C ₂₁	
Docosanol Docosyl sulphate Docosanoxy ethanol Docosanoic acid Octadecyl methacrylates	C ₂₂ , Behenyl alcohol Behenic acid	Docosanol, docosanoic acid

Note: Results are from laboratory scale experiments and naturally occurring hydrocarbons extracted from the particulate organic matter associated with the formation of water-repellant sands in Australia. Monolayer data is from Barnes (2008), water repellent sands data is from Franco et al., 2000).

Natural marine microlayer films consisting of 2 mg/L of lipid can dampen capillary waves and stay intact at wind speeds from 4 to 7 m/sec. Wind and wave action can break films into streaks, indicating that their composition is not even. As with monolayers, the degree of attraction between the aerial hydrophobic carbon chains determines the degree of condensation. If there are additional nonpolar hydrocarbons, a continuous hydrocarbon film of varying chain length may form, developing multilayers before breaking up into clusters or drops (Norkrans, 1980). Crystals may readily form as particulate matter if the film consists of mainly long-chain polar hydrocarbons.

1.3. Potential Interactions between Microlayers and Monolayers

The similarity between monolayer and microlayer chemical composition (Table 1) suggests that adverse interactions are likely to occur. The natural microlayer amphiphiles may act as 'impurities', producing holes that accelerate evaporative loss (Barnes & La Mer, 1962). The existence of microlayers on most water bodies also implies the presence of pre-adapted, monolayer-using bacteria. Phototrophic bacteria can use monolayer compounds such as hexadecanol as a substrate. The esterifying alcohols, farnesol, hexadecanol, hexadecenol, tetradecanol, phytol and octadecanol are all naturally synthesised components of bacteriochlorophyll (Glaeser & Overmann, 2003). More generally, bacteria use polyhydroxyalkanoic acids, triacylglycerols and wax esters as storage lipids (Uthoff et al., 2005). Wax esters are oxoesters of primary long-chain fatty alcohols and long-chain fatty acids. In nature, long-chain fatty acids and alcohols are widespread in plant species, and may constitute from two to 70 per cent of the cuticular wax composition of grasses (Dove & Mayes, 1991).

These waxy compounds are the precursors of microlayer compounds. Microlayer compounds derived from plant residues can adversely interact with artificial monolayers in three ways:

- As microbial substrates favouring the growth of microbes pre-adapted to degrade long-chain amphiphiles such as artificial monolayer compounds;
- As non-homologous amphiphilic hydrocarbons disrupting the orderly packing and/or forming holes in artificial monolayer films; and
- As chemically-reactive, humus-like compounds increasing photodegradation.

In this study, the water quality of microlayer and subsurface samples collected from six water bodies in Southeast Queensland was analysed. The water bodies were selected based on:

- The type of riparian vegetation in the immediate catchment (cleared agricultural land or woody remnant vegetation); and
- The main source of the water (overland flow, bore water, or pumped from a water course during peak flow).

Water quality was assessed as follows:

- Biochemical oxygen demand (BOD) was used to indicate the microbial reactivity of the water;
- Absorbance in the ultraviolet (UV) region was used to indicate the concentration of humus-like organic compounds; and
- Results for BOD and UV absorbance were compared with the potassium permanganate chemical oxygen demand (permanganate COD) and with published results of microlayer and subsurface water quality studies undertaken in the northern hemisphere.

Microlayer samples were also collected from water tanks that had been coated with three artificial surface films (two monolayer products and one thicker silicone film) to examine how the films affected the water quality of the microlayer. The microbial resilience of three artificial monolayer compounds was compared using bacterial species isolated from activated sludge. The microbes were selected on their ability to use the aromatic organic substrate phenol as an organic carbon source (Geng *et al* 2006).

2. Materials and Methods

2.1. Characterising Natural Microlayers on Local Water Bodies

2.1.1. Selection of Water Bodies and Sampling Methods

Six water bodies in Southeastern Queensland were sampled during November and December 2007 at the end of a long drought. One of the water bodies was sampled again, in July 2008, after rain had replenished the storage. Indicator oils were dropped onto the water surface to determine surface pressure (Goldacre, 1949). A dilution series of dodecanol dissolved in chloroform was dropped onto the water surface, starting with a concentration with the least tendency to spread until the next concentration equalled the spreading pressure of the water. The spreading pressure of the dodecanol dilution series was calibrated using a Langmuir trough (Barnes & Gentle, 2005).

For the first sampling, surface pressure was measured after applying 600 mL of microlayer sample to a water trough. For the second sampling, the surface pressure was measured *in situ*, by placing drops within a floating circular boom. Sampling was done at dawn or dusk when it was less windy. A 20.5 cm long x 20.0 cm wide plate sampler was made from 10 mm thick hydrophobic Teflon sheet (Larsen et al., 1974). Conical indents were drilled into the sheet, perforating the base of the plate with small holes 1 mm in diameter. Before sampling, all equipment was rinsed with 96 per cent methanol to remove lipid and microbial contaminants (Kostrzewska-Szlakowska, 2005), and air-dried.

The plate was firmly placed into the surface of the water to a depth just less than the thickness of the plate, and the microlayer sampled. The plate was removed vertically from the surface and the subsurface water allowed to drain. After this, the microlayer sample in the conical indents was collected by shaking the plate above a Teflon baking tray (Hillbricht-Ilkowska & Kostrzewska-Szlakowska, 2004). The plate was dipped repeatedly into minimally disturbed sections of the water surface until 500 mL was collected. A composite subsurface sample of 500 mL was collected from 5 to 10 mm below the surface using a glass bottle (Hatcher & Parker, 1974). For larger water bodies, both the leeward and the windward shores were sampled to determine if the direction of the prevailing wind affected the properties of the microlayer. Water samples were stored at 4°C and analysed within ten days of collection.

2.1.2. Biochemical Characterisation of the Water Samples

The pH of the microlayer and subsurface water samples was recorded using an Orion pH meter (model 710A). A portable TPS MC84 probe was used to record the electrical conductivity of the water. The chloride concentration of the samples was measured by silver nitrate titration, and the five-day BOD was also recorded using an Orion 860 dissolved oxygen meter (Eaton et al., 2005). The permanganate index of the water samples was measured by back-titrating heated, acidified water samples with 0.002 mol/L potassium permanganate (Rump, 1988). For samples with a chloride ion concentration of above 300 mg/L, 0.5 mL of 1.36 g/mL sodium hydroxide was added to each 100 mL sample instead of sulfuric acid. The water samples were filtered through glass microfibre filter paper before their absorbance at 253.7 nm (UV absorbance) was recorded in a spectrophotometer (matched quartz cuvettes, Cecil CE2021 spectrophotometer).

The enrichment factor of the microlayer was calculated by dividing the value of a given parameter recorded for the microlayer by the corresponding value recorded for the subsurface water (Estep et al., 1985). The Pearson correlation coefficient (Systat software package version 5) was calculated to investigate the relationship between the chemical and biochemical parameters recorded for the water samples.

2.2. Impact of Artificial Surface Films Applied to Potable Water on Water Quality

During April and May 2008, three evaporation control compounds were applied to polyvinyl chloride water troughs filled with potable tap water. The surface area of the troughs was 3 m² and they were 0.7 m deep. The compounds were a mixture of hydrated lime and the monolayer hexadecanol, a silicone oil surface film, and the monolayer compound monoM dissolved in hexane.

For the first trial, the compounds were applied at rates three times the commercial recommendations:

- 1 kg/ha for the hexadecanol mix re-applied every two days;
- 2 L/ha for the silicone oil re-applied after ten days ; and
- 50 g/ha for monoM re-applied after ten days.

Application rates for the second trial were the same, but both the hydrated lime-hexadecanol mix and monoM were applied every second day. The plate sampler (Section 2.1.1) was used to collect microlayer samples from each of the water troughs,

including a control trough to which no evaporation control compound had been added. Microlayer samples of 500 mL were collected and analysed for pH, electrical conductivity and BOD5 using the methods described in section 2.1.2.

2.3. Screening Artificial Monolayer Compounds for Microbial Resilience

2.3.1. Isolation of Acinetobacter and Pseudomonas cultures

A sample of 10 mL activated sludge from a municipal wastewater treatment plant was used to select phenol-degrading microbes. The activated sludge was inoculated into 90 mL of a mineral salts broth (MS broth Bouchez-Naitali et al., 1997) to which 500 mg/L of phenol had been added (MP500 broth) as the sole organic carbon source (Geng et al., 2006). Cultures were incubated at room temperature (10 to 25°C) on an orbital shaker (120 rpm) until the change in optical density in a spectrophotometer set at a wavelength of 600 nm indicated good microbial growth. A sample of 10 mL was re-inoculated twice more into 90 mL of the mineral salts medium supplemented with phenol to select for phenol-degrading bacteria. After the third selective culturing, bacteria were isolated by streaking inocula onto trypticase-soy agar and characterised by using the Gram stain and light microscopic observation.

Pure cultures of bacteria were re-inoculated into the mineral salts phenol medium and incubated until the optical density of the culture medium was between 0.6 and 0.7 optical density units (ODUs) recorded at 600 nm wavelength (OD_{600} Gerhardt *et al* 1994). Dilution plate counts using peptone water and trypticase-soy agar were used to count the viable cell population in the culture medium grown to 0.7 ODU. The plates were incubated at 25°C until viable colonies could be counted. Inocula prepared to this density in MP500 broth were used to compare the resilience of artificial monolayer compounds added as the sole organic carbon source to the mineral salts medium.

2.3.2. Microbial Degradation of Artificial Monolayer Compounds

A C18 compound with a different headgroup (monoM) dissolved in acetone or 5 mL of stock solutions of the fatty alcohol monolayer compounds hexadecanol (C16OH) or octadecanol (C18OH) was added to the base of sixteen sterilised 250 mL conical flasks. These flasks were placed overnight on an orbital shaker (120 rpm) to coat the lower internal surface to a height of 1.5 cm (Jinxia Liu et al., 2007). To produce a final monolayer concentration of 1 mM as the sole source of organic carbon for microbial growth, 20 mL of sterilised MS broth (Bouchez et al., 1997) was added to each flask. The flasks were inoculated with 2 mL of either Acinetobacter or Pseudomonas isolates grown in MP500 broth to an OD_{600} of between 0.6 and 0.7 ODU. Twelve inoculated

MS monolayer broth cultures were incubated at 25 °C on a heated orbital shaker at 120 rpm for up to four days. Four uninoculated control flasks were prepared as above on the fourth day, and incubated for 24 and four hours on the heated shaker for series one and two respectively, before destructive sampling.

After two, three and four days of incubation, four replicate flasks of the inoculated broth cultures were destructively sampled by adding 20 mL of chloroform to recover any monolayer compound left in the MS broth. After chloroform was added, the flasks were placed on magnetic stirrers for 20 minutes before the fluid was decanted into a separation funnel. The chloroform and interfacial phases were collected separately in glass vials and were stored frozen before being analysed on a gas chromatograph.

The concentration of monolayer compound extracted in the chloroform phase was measured on a Shimatsu GC-2010, GMS QP 2010 Plus using a RTX5 column 30 m x 0.25µm internal diameter and 0.25µm layer thickness. The injection temperature was 270°C, initial column temperature was 130°C, ramped to 200°C at 20° per minute, zero hold, 200-300°C at 10° per minute, zero hold, and a total run time of 13.5 minutes. Helium was the mobile phase, with a linear velocity of 52.6 cm sec⁻¹, total flow of 102.4 ml min⁻¹, split ratio of 50:1, ion source and interface temperatures of 250°C, with the mass spectrophotometer on E1 setting. A dilution series of 1.0 mM, 0.5 mM, 0.3 mM, 0.2 mM and 0.1 mM of each monolayer compound dissolved in chloroform was prepared and injected into the gas chromatograph to provide a concentration standard for each microbial degradation trial.

2.3.3. Quantifying the Microbial Population Using the Monolayer Compounds in Broth Culture

The aqueous phase of the broth cultures decanted from the separation funnel was used to prepare a cell extract. Samples were centrifuged immediately for five minutes at 3,000 rpm to remove cellular fragments and any residual chloroform. A pipette was used to remove 10 mL of the supernatant, which was placed in a glass vial and stored frozen before analysis. After thawing, 5 mL of the supernatant was gently mixed for two minutes with an equal volume of a phenol:chloroform:isoamyl alcohol solution (25:24:1) and centrifuged at 3,000 rpm for five minutes to remove cellular debris (Ausubel et al., 1995). A 0.8 mL sample of the supernatant containing the DNA and RNA was pipetted off, placed in a microcentrifuge tube and gently mixed for two minutes with 1.2 mL of isopropanol. The microcentrifuge tube was spun at 3,000 rpm for thirty seconds to pelletise the DNA. The supernatant was drained off, in droplets, to avoid resuspending the pellet. Two 1 mL aliquots of 80 per cent ethanol were pipetted into the centrifuge

tube to precipitate the DNA present in the pellet, and the supernatant was drained off in droplets. The centrifuge tubes were inverted to allow the DNA pellet to air dry for 30 minutes.

The DNA pellet was re-suspended by adding 50 mL of Tris EDTA buffer to each tube (Ausubel et al., 1995). The tubes were mixed gently and left overnight in a refrigerator to re-suspend. The DNA suspension was further mixed using a vortex and centrifuged for ten seconds at 3,000 rpm. Three microlitres of the purified DNA solution was placed into an Implen Nanophotometer cuvette and the absorbance of the sample recorded using wavelengths of 230, 260 and 280 nm. The DNA concentration in each suspension was calculated using the relationship of 1.0 absorbance units at 260 nm corresponding to 50 µg of double stranded DNA (Brown, 1990). The purity of the DNA sample was checked by calculating the ratio of the absorbances at 260 and 280 nm. Ratios of less than 1.8 were considered contaminated, with either protein or phenol.

3. Results

3.1. Water Quality Attributes of Microlayers on Southeast Queensland Water Bodies

The water in the university agricultural plot (AgPlot) tank was expected to be of poor quality as the result of a history of repeated application of sodium hypochlorite. This was reflected in the very high electrical conductivity (EC) and chloride ion concentration and in the high pH (Table 2). However, other natural water supplies also had high concentrations of chloride and high ECs. The Brimblecombe ringtank was supplemented during the drought with saline bore water, and the catchment of Cooby Dam includes sodic, alkaline soils (Table 3). The water body that had the lowest chloride concentration and EC was the Alderton ringtank, however, because bentonite clay was in the soil underlying the catchment of Dogwood Creek, the water was extremely turbid (Figure 1e). The clay particles were so fine that they passed through the glass fibre filter paper. No UV absorbance results were possible for this sample. As expected, the electrical conductivity was positively correlated with the chloride ion concentration (Pearson Correlation of 0.94, $P < 0.001$).

Table 2. Water quality attributes of the six water storages.

	pH		Electrical conductivity $\mu\text{S/m}$		Chloride ion concentration mg/l	
	subsurface	microlayer	subsurface	microlayer	subsurface	microlayer
Agricultural plot tank	9.1	9.1	8240	8585	1569	1610
Narda Lagoon	8.4	8.2	260	280	33	37
Laidley Lake Dyer	8.1	8.2	1760	1720	572 W 571 L	570 W 574 L
Laidley						
Brimblecombe ring tank	8.4	8.4	5855	5910	1789	1942
Cooby Dam,	8.9 W	8.8 W	2368 W	2467 W	676 W	691 W
Meringandan	8.7 L	8.8 L	2417 L	2501 L	681 L	701 L
Alderton ring tank, Yulabilla	7.8 W 8.0 L	7.8 W 7.8 L	212 W 207 L	218 W 211 L	32 W 33 L	36 W 39 L

Note: Water storages sampled during October and November 2007. For the larger storages, subsurface and surface layer (microlayer) samples were taken from the windward and lee shores (W and L respectively). An Orion meter (model 710A) was used to measure pH, and a TPS MC84 meter was used to measure conductivity. The concentration of chloride was measured by titrating with silver nitrate (Eaton et al., 2005).

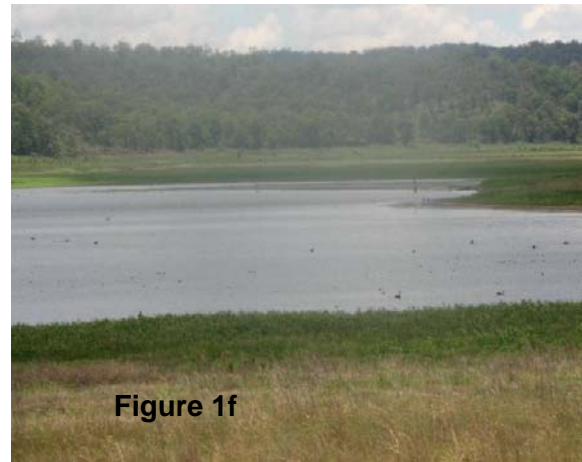
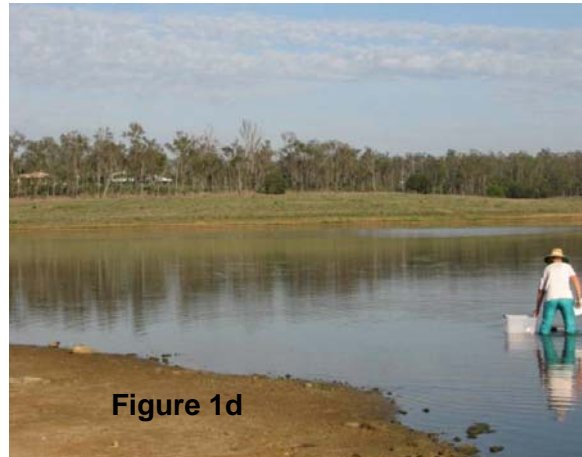


Figure 1. The six water bodies located in Southeast Queensland.

Note: Water bodies sampled during October and November 2007. A Larsen plate was used to sample the water surface and a glass bottle for the subsurface water. Figure 1a is the experimental water tank at the University of Southern Queensland, Toowoomba; Figure 1b, Narda Lagoon at Laidley; Figure 1c, the irrigation ring tank at Forest Hill; and Figure 1d, the recreational and irrigation storage dam Lake Dyer, near Laidley. Figure 1e is the recently built ring tank irrigation storage at Yulabilla near Condamine, and Figure 1f is the municipal water storage Cooby Dam at Meringandan, near Toowoomba.

Table 3. Description of the water bodies sampled during the drought in October and November 2007.

Name	Water source	Surface area at full capacity	Riparian Vegetation	Function
University agricultural plot water tank, Toowoomba	Reticulated town water, rainwater		None	Experimental site for monolayer testing
Narda Lagoon, Laidley	Overland flow from rural residential land and a sawmill	1 ha	Eucalypts, acacias on fringe, grasses	Storm water retention, public park
Lake Dyer (Bill Gunn Dam), Laidley	Overland flow and peak flow off-take from Laidley Creek	100 ha	Sparse eucalypt woodland with grassy understorey	Irrigation storage, recreation
Brimblecombe ring tank, Forest Hill	Peak flow off-take from Sandy Creek, and bore water	0.5 ha	Grassland and intensive cropping land	Farm irrigation storage
Cooby Dam, Meringandan	Cooby Creek, overland flow	306 ha	Eucalypt woodland on sodic subsoil	Municipal water supply for Toowoomba
Alderton ring tank, Yulabilla	Peak flow off-take from Dogwood Creek	45 ha	Eucalypt woodland, bentonite clay subsoil	Recently constructed farm irrigation storage

Note: Sites were selected on the basis of the water source, the nature of the vegetation immediately around the water body (riparian zone) and the purpose the water storage was built and/or used for. (See photos in Figure 1.)

The high algal growth in the agricultural plot tank (Figure 1a) was reflected in the very high BOD values for the subsurface and microlayer samples (Table 4). These BOD values were the highest recorded for all six water bodies, but the permanganate index and UV absorbance values were lower than Narda Lagoon, Lake Dyer and Alderton ringtank. These three water bodies share the characteristics of having a treed canopy in the catchment area (Figure 1). Cooby Dam's catchment is also wooded, but the total volume of water is greater (Table 3). This may dilute the humified organics contributing to these properties.

The presence of plant-wax-coated suspended clay particles in the Alderton water sample may explain the high permanganate index, as there was very little difference between the microlayer and subsurface values (Table 4). As expected, the permanganate index was positively correlated with UV absorbance and negatively correlated with the chloride concentration (Table 5) when the Pearson correlation was used to analyse the data. Unexpectedly, the chloride concentration was positively correlated with the BOD, but as the probability was relatively low ($P = 0.027$, $n = 15$) this may not be biologically meaningful.

The surface pressures recorded for the water storages ranged from a low of less than 7 mN m⁻¹ to 15.6 mN m⁻¹ (Table 6). While these values are similar to the range of surface pressures recorded for marine microlayers, of 1.00 to 13.6 mN m⁻¹ (Frew & Nelson, 1992), they are well below the spreading pressures recorded for artificial monolayer compounds that are known to suppress evaporative loss. Hexadecanol and octadecanol have spreading pressures of 39 and 35 mN m⁻¹ (Barnes, 2005). The difference in the surface pressures for the 2007 and 2008 sampling times suggest that seasonal variation may occur.

Table 4. Water quality attributes of the six water storages.

	Biochemical Oxygen Demand (BOD5) mg/l		Permanganate Chemical Oxygen Demand mg/l		Absorbance at 253.7 nm	
	subsurface	microlayer	subsurface	microlayer	subsurface	microlayer
Agricultural plot	7.7	11.9	10.8	21.2	0.27	0.31
Narda Lagoon	1.1W	10.3 L	10.9 L	25.4 W	0.35 W	0.47 W
Laidley	1.3 L			23.8 L	0.36 L	0.45 L
Lake Dyer	4.8 W	6.2 W	13.7 W	12.2 W	0.55 W	0.61 W
Laidley	5.2 L	6.4 L	13.6 L	14.4 L	0.58 L	0.61 L
Brimblecombe ring tank	2.3	9.8	6.0	8.9	0.07	0.07
Cooby Dam,	2.2 W	3.5 W	6.6 W	7.9 W	0.12 W	0.15 W
Meringandan	2.4 L	5.3 L	6.9 L	8.0L	0.13 L	0.14 L
Alderton ring tank, Yulabilla	1.8 W 1.8 L	1.5 W 2.1 L	47.2 W 47.3 L	53.4 W 51.4 L	na	na

Note: Water storages sampled during October and November 2007. For the larger storages, subsurface and surface layer (microlayer) samples were taken from the windward and lee shores (W and L respectively). The biochemical oxygen demand incubated for 5 days was measured using an Orion oxygen meter. The chemical oxygen demand was calculated from the permanganate index. Water samples were filtered through glass microfibre paper before to reading their absorbance in the ultraviolet spectrum with a Cecil CE2012 spectrophotometer.

Table 5. Pearson correlation matrix indicating the relationships between the permanganate index, absorbance in the ultraviolet spectrum, chloride ion concentration and biochemical oxygen demand.

	Permanganate Index mg/l	Absorbance at 253.7 nm	Chloride ion mg/l	BOD 5 mg/l
Permanganate Index mg/l	1.000			
Absorbance at 253.7 nm	0.597 *	1.000		
Chloride ion mg/l	-0.584 *	-0.524 ns	1.000	
BOD 5 mg/l	-0.290 ns	0.146 ns	0.521 *	1.000

Note: Readings recorded for the six water bodies sampled during October and November 2007. The star indicates that the correlation value is statistically significant at P<0.05 (n = 15). The abbreviation 'ns' indicates that the correlation value is not statistically significant.

Table 6. Water quality attributes of the three water storages.

	pH		Electrical conductivity $\mu\text{S/m}$		Surface pressure mN m^{-1}
	subsurface	microlayer	subsurface	microlayer	
Narda Lagoon Laidley 2007	8.4	8.2	260	280	11 mNm^{-1}
Narda Lagoon Laidley 2008	7.5	7.6 W 7.5 L	202	211 W 219 L	< 7 mNm^{-1}
Lake Dyer Laidley 2007	8.1	8.2	1760	1720	15.6 in reeds <5.9 open w
Lake Dyer Laidley 2008	7.49	7.44	371	367	11 in reeds <5.9 open w
Alderton ring tank, 2007	7.8 W	7.8 W	212 W	218 W	na
Alderton ring tank, 2008	8.0 L	7.8 L	207 L	211 L	

Note: water storages sampled during October and November 2007, and again during May to July 2008. Subsurface and surface layer (microlayer) samples were taken from the windward and lee shores (W and L respectively). An Orion meter (model 710A) was used to measure pH, and a TPS MC84 meter was used to measure conductivity. Surface pressure was estimated using a dilution series of dodecanol indicator oils.

Table 7. Water quality attributes of the six water storages.

	Biochemical Oxygen Demand (BOD5) mg/l		Permanganate Chemical Oxygen Demand mg/l		Absorbance at 253.7 nm	
	subsurface	microlayer	subsurface	microlayer	subsurface	microlayer
Narda Lagoon Laidley 2007	1.1W 1.3 L	10.3 L	10.9 L	25.4 W 23.8 L	0.35 W 0.36 L	0.47 W 0.45 L
Narda Lagoon Laidley 2008	4.4	5.1 W 8.7 L	54.55	99.31 W 98.72 L	1.10	1.04 W 1.00 L
Lake Dyer Laidley 2007	4.8 W 5.2 L	6.2 W 6.4 L	13.7 W 13.6 L	12.2 W 14.4 L	0.55 W 0.58 L	0.61 W 0.61 L
Lake Dyer Laidley 2008					0.207	0.220
Alderton ring tank, 2007	1.8 W 1.8 L	1.5 W 2.1 L	47.2 W 47.3 L	53.4 W 51.4 L	na	na
Alderton ring tank, 2008						

Note: Water storages sampled during October and November 2007, and again from May to July 2008. For the larger storages, subsurface and surface layer (microlayer) samples were taken from the windward and lee shores (W and L respectively). The biochemical oxygen demand incubated for 5 days was measured using an Orion oxygen meter (model 860). The chemical oxygen demand was calculated from the permanganate index. Water samples were filtered through glass microfibre paper prior to reading their absorbance in the ultraviolet spectrum with a Cecil CE2012 spectrophotometer.

3.2 Evidence of Microlayers on Southeast Queensland Water Bodies

Evidence of a microlayer was most obvious at Narda Lagoon with streaks of small particles formed in bands observed at the lee shore. Surface films were also observed on the lee shore of Cooby Dam and Lake Dyer, but not to the same extent as at Narda lagoon. Turbidity and wave action during sampling at Alderton made it hard to see any surface film. Using the permanganate index, evidence of enrichment in the microlayer was most obvious for Narda Lagoon, the university agricultural plot tank and the Alderton ring tank (Figure 2). However, using the BOD results, all of the water bodies except for the Alderton ring tank had an enriched microlayer. The degree of enrichment was as high as 9.8 for BOD and 2.3 for permanganate index (Narda Lagoon, see Table 8). Enrichment for BOD, permanganate index and UV absorbance ranged between 1.05 and 2.2 for the other water bodies. There was very little evidence of enrichment for chloride, EC and pH in any of the six water bodies.

Results for UV absorbance for the water bodies sampled in this Australian study are comparable to the humic lakes studied in Northern Europe (Figure 4). After calculating the chemical oxygen demand from the permanganate index, results for the Australian water bodies are higher, with only the Cooby Dam and Brimblecombe ring tank lower than the Norwegian samples (Figure 5). Enrichment values for UV absorbance, the permanganate index and BOD₅ varied a lot between the 2007 and the 2008 sampling (Table 8), suggesting that the physical and chemical attributes of natural microlayers may vary with the season. For the Narda Lagoon samples, reduced enrichment values for the 2008 sampling were due to a big increase in the permanganate index for the subsurface water (Table 7). Presumably this coincided with the increase in leaf litter and plant debris washed into the lagoon during heavy rain in the late summer of 2007, when the water level was replenished to full capacity.

Table 8. Enrichment factors calculated as the ratio of the microlayer value divided by the subsurface value of six water quality attributes.

	Enrichment pH	Enrichment EC	Enrichment Chloride	Enrichment BOD5	Enrichment Mn Index	Enrichment Absorbance 253.7 nm
Agricultural plot	1.00	1.04	1.03	1.54	1.97	1.15
Narda Lagoon	0.98	1.08	1.13	9.81	2.32 W 2.18 L	1.34 W 1.24 L
Laidley 2007				1.18 W 2.01 L	1.82 W 1.81 L	0.94 W 0.90 L
Laidley 2008						
Lake Dyer	0.99	0.98	1.00 W 1.01 L	1.29 W 1.20 L	0.90 W 1.06 L	1.06 W 1.11 L
Brimblecombe ring tank	1.00	1.01	1.09	4.20	1.47	1.05
Cooby Dam, Meringandan	0.99 W 1.00 L	1.04 W 1.04 L	1.02 W 1.03 L	1.62 W 2.23 L	1.20 W 1.17 L	1.23 W 1.06 L
Alderton ring tank, Yulabilla	1.01 W 0.98 L	1.03 W 1.02 L	1.12 W 1.19 L	0.83 W 1.18 L	1.13 W 1.09 L	na

Note: Recorded for the six water storages sampled during October and November 2007. Subsurface and surface layer (microlayer) samples were taken from the windward (W) and lee shores (L) of larger storages

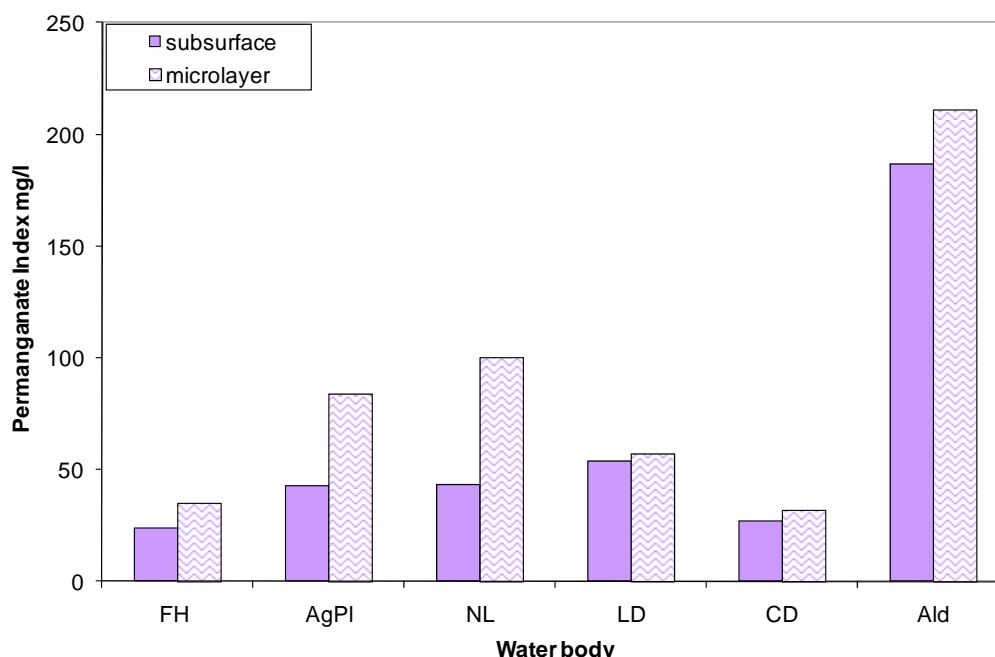


Figure 2. The permanganate index recorded for surface and subsurface water samples collected from the six water storages during October and November 2007.

Note: Solid bars are the subsurface samples collected 5 to 10mm below the surface, wavy bars are the surface layer. The water storages are the Brimblecombe ringtank at Forest Hill (FH), the USQ agricultural plot tank (AgPI), Narda Lagoon (NL) and Lake Dyer (LD) near Laidley, Cooby Dam municipal water storage for Toowoomba (CD), and the Alderton irrigation ring tank (Ald).

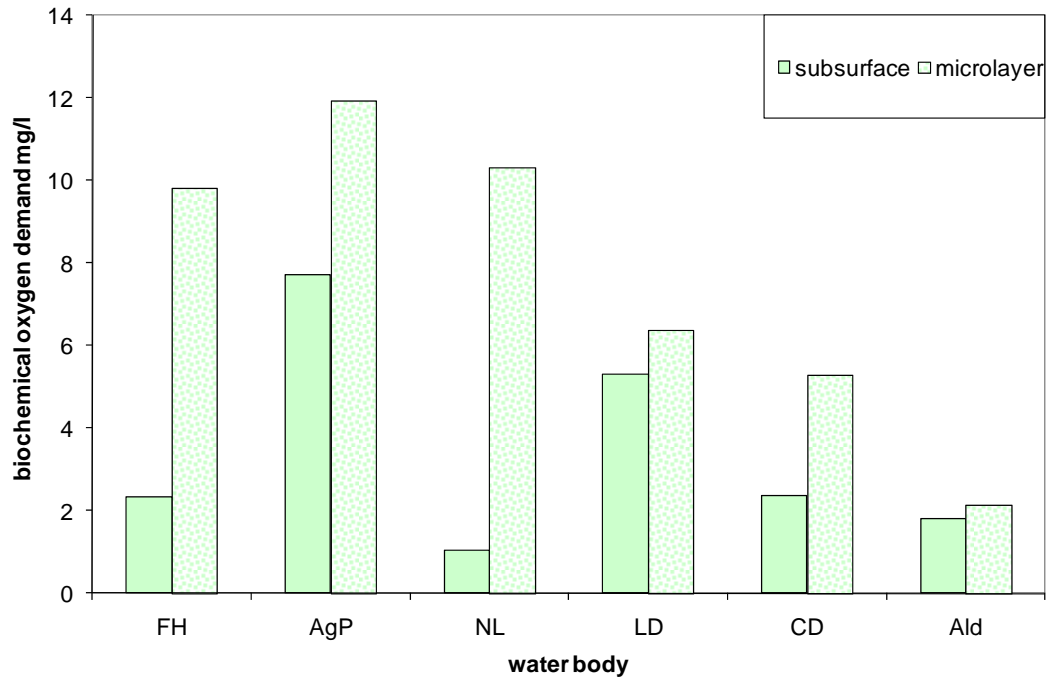


Figure 3. The BOD5 readings for surface and subsurface water samples collected from the six water storages during October and November 2007.

Note: Solid bars are the subsurface samples collected 5 to 10 mm below the surface, stippled bars are the surface layer.

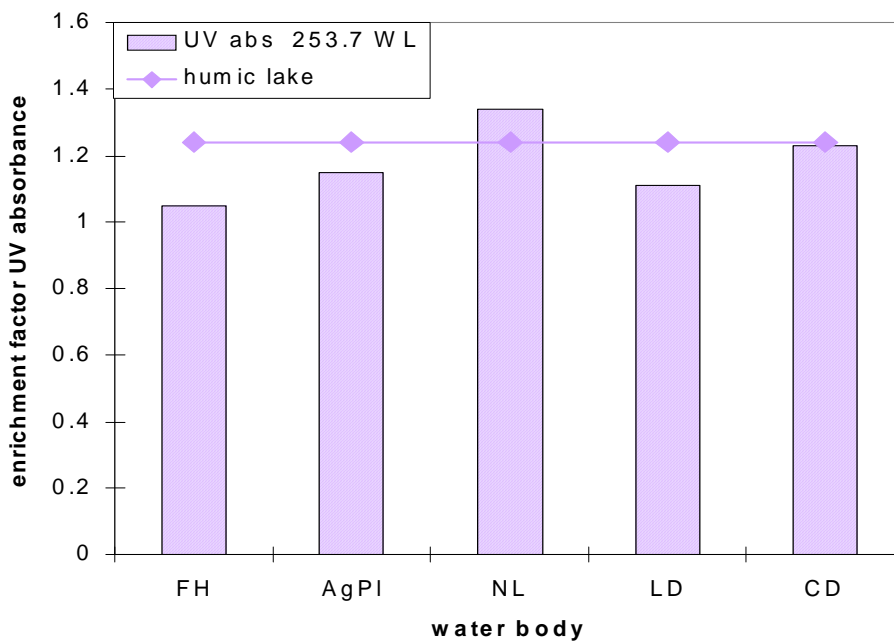


Figure 4. The enrichment factors for absorbance in the ultraviolet region, recorded for filtered water samples.

Note: Samples taken from five of the six water bodies during October and November 2007. The Alderton ring tank is not included. The horizontal line is the highest absorbance value recorded for water sampled from humic lakes in Poland (data from Kostrzevska-Szlakowska, 2005).

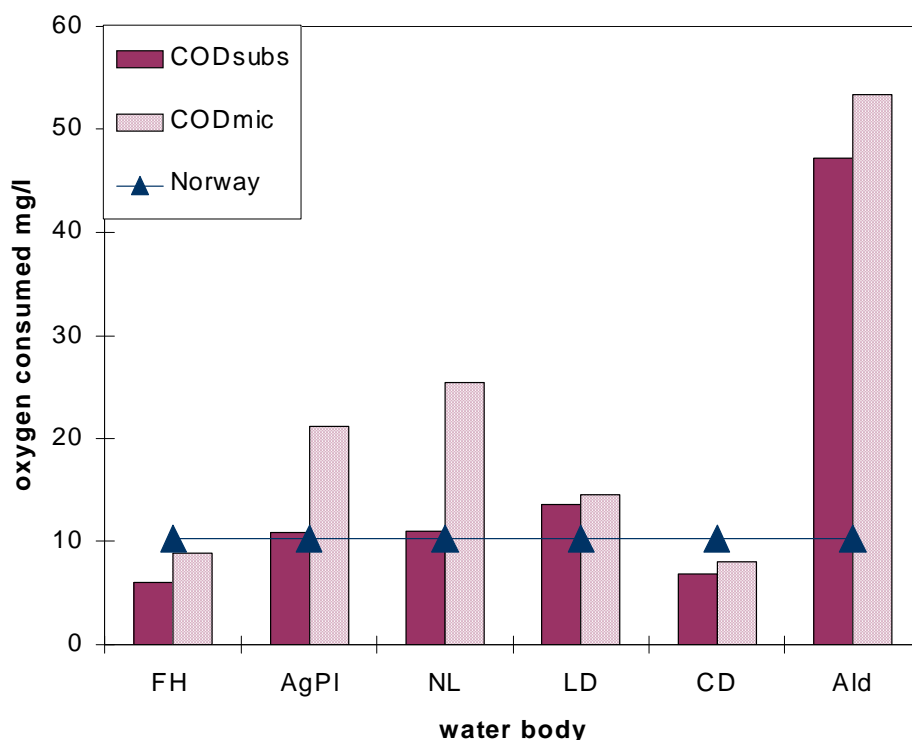


Figure 5. A comparison of the permanganate chemical oxygen demand recorded for the subsurface (solid bar) and surface layers (wavy bar) of water sampled from the six water storages during October and November 2007.

Note: The horizontal bar is the highest permanganate chemical oxygen demand value recorded for humic lakes sampled in Norway (data from Hessen, 1985).

3.3. Impact of Artificial Surface Films on the Quality of Municipal Potable Water

Sampling immediately after application showed that all three films reduced the surface tension of the water for both trials.

In the first trial, twenty dips of the Larsen plate sampler were needed to produce about 400 mL of microlayer sample for the tank with no surface layer applied. For all three surface layer treatments, more than fifty dips were required to produce 400 mL of sample. By day ten of the first trial the effect on surface tension had decreased; twenty-five dips were required for the tank with no surface layer applied, and only twenty-five dips for the other treatments. Day ten sampling was done during light, drizzling rain. The rainbow-slick effect of the silicone oil on the water surface was most obvious, but there was some evidence of a surface film in the hexadecanol treatment with silver eddies moving away from the plate when dipped.

In the second trial, thirty-five dips produced 450 mL of day zero sample from the tank with no surface treatment applied, but seventy dips were needed for the silicone oil and

the monoM monolayer compound. In contrast, and despite the same amount of product being applied, the hexadecanol and hydrated lime product required only fifty-five dips to produce 450 mL. With fewer plate dips required to collect the hexadecanol hydrated lime product suggests that there was less monolayer compound in the sample applied in tank trial two. The BOD results support this conclusion, with the day zero reading in trial one much higher than the reading for day zero in trial two (34.5 mg/L and 8.3 mg/L respectively, Table 9). The BOD readings for the control tanks to which no film was applied were also lower in trial two, but the reduction is not as great as for the hexadecanol film. BOD results for monoM for day 0 are similar for both trials (21.0 and 29.7 mg/L for trials one and two respectively), but the result for trial one is 60 per cent lower than the value recorded for the hexadecanol film.

The high BOD result for the day 0 hexadecanol film sample indicates how readily microbes use the compound as an organic carbon source. However, the very alkaline pH value of 10.2 associated with the hydrated lime carrier may adversely affect aquatic life. The pH at day zero in trial two was higher (10.9, Table 9), suggesting that the ratio of hydrated lime to monolayer compound was higher in trial two than for trial one. The extreme pH appears to be a transient effect, reducing to 8.8 and 9.1 by day ten, two days after a repeat application. None of the compounds appeared to change the electrical conductivity of the surface interface (Table 9), and none of the compounds absorbed light to any extent in the ultraviolet region (data not included).

The BOD results for the silicone oil are of concern (Table 9). In trial one, the results were off scale for both the day zero and the day ten sampling, suggesting that the oil is acting as an oxygen scavenger. The lowest dilution factor used in trial one was 0.1, reduced to 0.017 in trial two. Even at a dilution of 0.017, immediately after the film was applied (day zero), the scavenging effect was enough to remove all dissolved oxygen. BOD results for day ten in trial two are confusing, with all values except the no-film control well below the expected values.

The result for monoM was on scale, but very low given that in this trial the compound was re-applied every two days. The range of dilutions used for the hexadecanol film in trial two were reduced to 0.1 to 0.017, on the basis that in trial one all of the oxygen was consumed when the sample was diluted within the range of 0.25 to 0.1. However, by day ten, the amount of hexadecanol remaining in the sample was too low to register any demand for oxygen, even at a dilution of 0.1. Using the same reduced dilution scale, a result of 10.5 mg/L was obtained for the silicone oil. These results suggest that when sampling on day ten of trial two, a much smaller concentration of the artificial

surface films was collected. Despite a smaller film sample being collected, the silicone oil still registered a high oxygen demand.

Samples were taken within five minutes of a passing sun-shower. It is possible that the larger, relatively colder droplets of water of the sun-shower may have submersed or dispersed the artificial surface films (Green & Houk, 1979). The only other factor that differed between the two trials was the density of insect cadavers present on the surface of the artificial films. Trial two was conducted during a full moon. The reflective silicone oil attracted the most insects, nine times more than on the control tank with no applied film. The density of insects on the monoM film was only three times that of the control. Most of the insect cadavers on the hexadecanol film were dismembered and fragmented, making a density comparison difficult. Either the hydrated lime, or the increased microbial activity stimulated by hexadecanol, or both may be responsible for this effect. In the absence of objective data, no further interpretation can be made.

Table 9. Water quality results for the microlayer sampled from tanks filled with potable municipal water onto which three artificial surface films were applied.

Table 9a. Results for tank trial one.

Tank trial 1	Day 0			Day 10		
	pH	EC μ S	BOD 5 O ₂ mg/l	pH	EC μ S	BOD 5 O ₂ mg/l
No film applied	7.9	1058	6.09	8.9	1202	2.3
Silicone oil	7.2	1124	Off scale	8.6	1259	Off scale
C16OH with hydrated lime	10.2	984	34.51	8.8	1222	Off scale
monoM monolayer in hexane	8.1	1054	21.0	8.7	1163	5.17

Table 9b. Results for tank trial two.

Tank trial 2	Day 0			Day 10		
	pH	EC μ S	BOD 5 O ₂ mg/l	pH	EC μ S	BOD 5 O ₂ mg/l
No film applied	8.2	794	2.8	8.2	995	2.4
Silicone oil	8.3	910	Off scale	8.3	1101	10.5
C16OH with hydrated lime	10.9	830	8.3	9.1	1043	Below limit of detection
monoM monolayer in Hexane	8.3	918	29.7	8.5	1047	2.5

Note: The surface-active compounds were a silicone oil, the monolayer compound hexadecanol mixed with a hydrated lime carrier, and the monolayer product monoM dissolved in hexane. The microlayer was sampled with a Larsen plate, immediately after the compounds were applied (day 0), and 10 days after the first application. In trial 1 both monoM and the silicone oil product were applied once (day 0), but the hexadecanol product was applied every second day. In trial 2, both the monoM and hexadecanol product were applied every second day. Sampling on day 10 was 2 days after the last application.

3.4. Screening Artificial Monolayer Compounds for Microbial Resilience

3.4.1. Enrichment and Quantification of *Acinetobacter* and *Pseudomonas* Inocula

Only *Pseudomonas* and *Acinetobacter* strains present in the activated sludge sample could use phenol as a substrate. Morphologically, both isolates produced Gram negative rods, but the cells of isolates, putatively identified as *Acinetobacter*, did not decolour readily when alcohol was added. The *Acinetobacter* cells were also much shorter and fatter than the thinner, longer cells putatively identified as *Pseudomonas* (Towner 1992). The *Acinetobacter* strains grew faster than the *Pseudomonas* strains, reaching an OD₆₀₀ of 0.6 to 0.7 ODUs in 2.5 days while *Pseudomonas* took more than 3 days. Dilution plate count results indicated that an OD₆₀₀ of 0.7 ODUs corresponded to a microbial population of 90 x 10⁶ colony forming units of *Acinetobacter* per mL of MP500 broth. MP500 broth cultures of *Acinetobacter* conforming to an OD₆₀₀ of 0.7 ODUs were used for all microbial degradation trials.

3.4.2. Microbial Degradation of Artificial Monolayer Compounds

After 20 mL of the mineral salts medium was added to each conical flask, the concentration of the monolayer compounds was adjusted to equal 1 mM after 2 mL of MP500 inocula were added. However, only 20 mL of chloroform was added to the 22 mL of microbial broth when the remaining monolayer compounds were extracted. Accordingly, the concentration of the monolayer compounds in the controls for the first microbial degradation trials were routinely above 1 mM (an average of 1.1 for trial 1, Figures 6, 7 and 8). Timing constraints in the second series of hexadecanol and octadecanol trials resulted in the uninoculated controls being incubated for only two instead of four hours. Halving the incubation time resulted in fewer monolayer compounds being recovered from the broth. Instead of 1.1 mM, the concentration dropped to 0.86 for hexadecanol, 0.81 for octadecanol and 1.01 for monoM, reductions of 22, 26 per cent and 8 per cent respectively. This reduced recovery is most evident in the second octadecanol and monoM trials (Figures 7 and 8), where the concentration of the monolayer compounds appear to increase after two days incubation.

Despite the lower recovery of monolayer compound in the second series, analysis of variance results for both hexadecanol trials indicate that the length of incubation after inoculation greatly reduced monolayer recovery ($P < 0.001$ for both trials). Incubation after inoculation also greatly affected the recovery of octadecanol ($P = 0.004$ for the first trial and 0.003 for the second) and monoM ($P = 0.004$ for the first trial and < 0.001 for the second). A comparison of the recoveries of the monolayer compounds in

Figure 9 indicates that hexadecanol degrades much more rapidly than octadecanol, with monoM the most resilient.

Data for the recovery of DNA from the monolayer broth media is only available for series two. Using hexadecanol as the microbial substrate, the rise in the concentration of DNA is inversely proportional to the decline in the monolayer concentration (Figure 10). The negative correlation between DNA and hexadecanol is significant ($P < 0.001$), with a Pearson correlation coefficient of -0.886 . Figure 11 indicates that as the concentration of octadecanol decreases there is a corresponding increase in DNA up to and including three days of incubation. However, after the third day the concentration of DNA drops. This inconsistency between the concentration of octadecanol and DNA is reflected in the Pearson correlation result. The correlation coefficient is negative as expected (-0.405), but the result is not significant ($P = 0.120$). The decline in monoM concentration over the length of incubation is very shallow, as is the corresponding increase in the concentration of DNA (figure 12). As expected, the Pearson correlation coefficient is small and negative (-0.058), but is not statistically significant ($P = 0.830$).

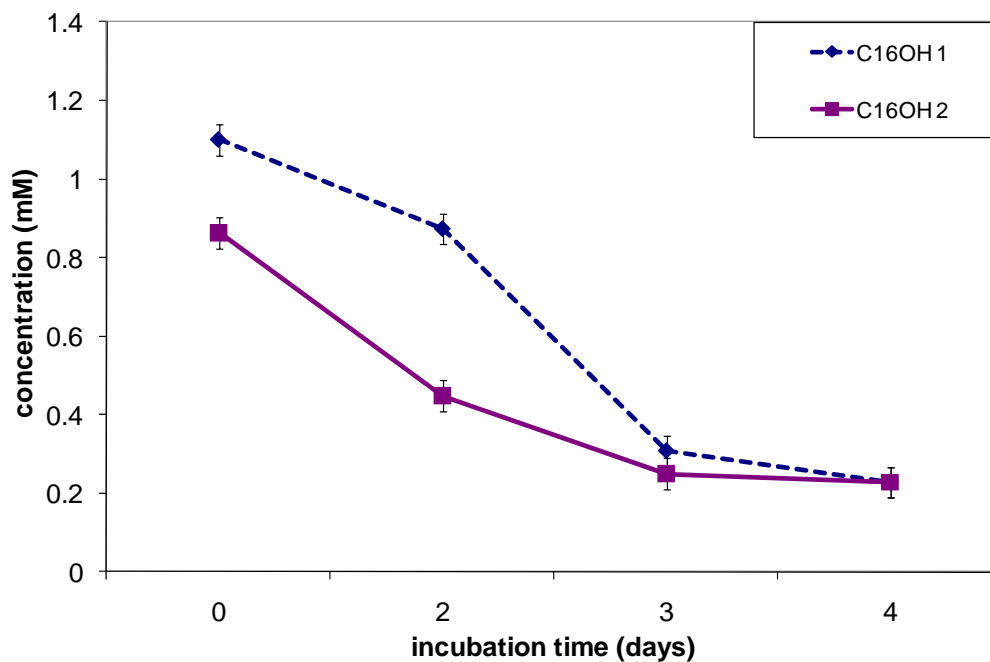


Figure 6. The concentration of hexadecanol (C16OH) remaining in the mineral salts medium inoculated with *Acinetobacter* incubated for up to four days.

Note: C16OH was the only organic carbon source in the broth medium. The uninoculated controls were incubated for four hours in trial one, but only two hours in trial two. Least significant means are shown plus and minus the standard errors.

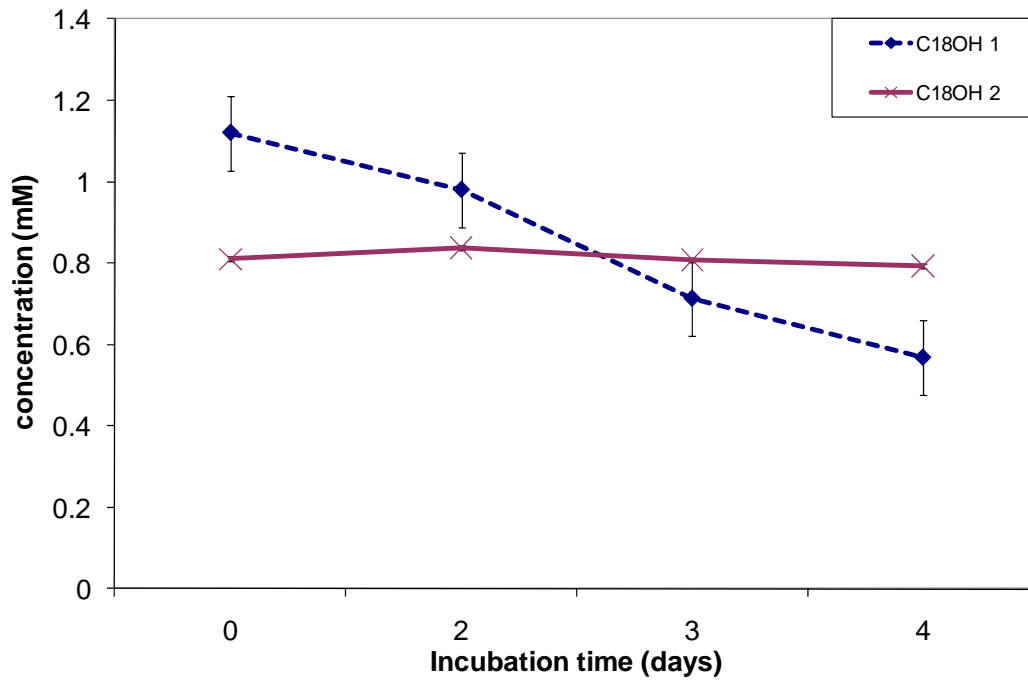


Figure 7. The concentration of octadecanol (C18OH) remaining in the mineral salts medium inoculated with *Acinetobacter*, incubated for up to four days.

Note: C18OH was the only organic carbon source in the broth medium. The uninoculated controls were incubated for four hours in trial 1, but only two hours in trial 2. Least significant means are shown plus and minus the standard errors.

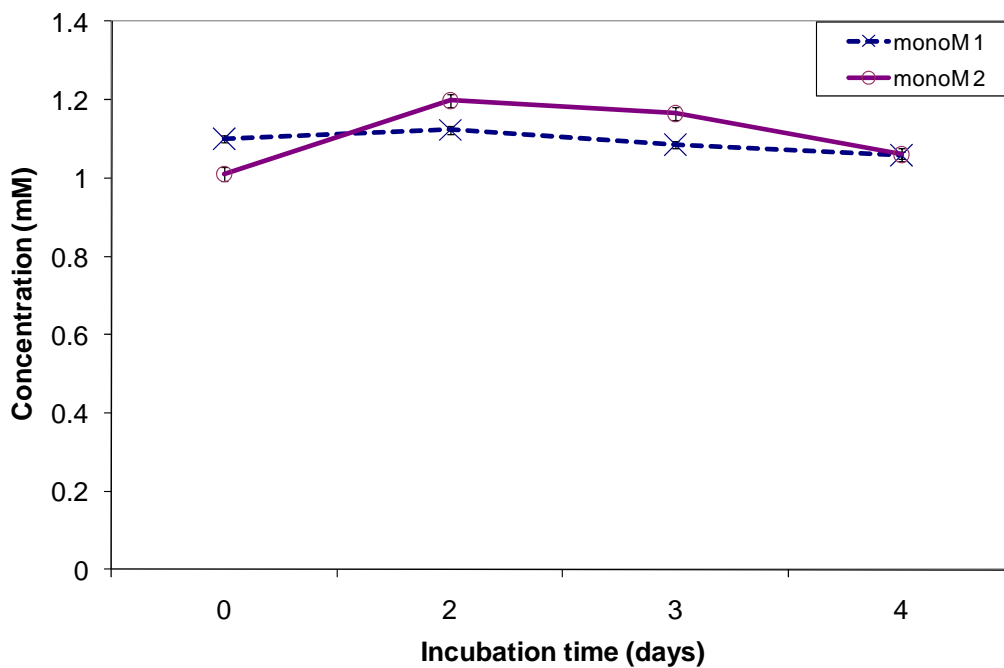


Figure 8. The concentration of monoM remaining in the mineral salts medium inoculated with *Acinetobacter*, incubated for up to four days.

Note: MonoM was the only organic carbon source in the broth medium. The uninoculated controls were incubated for only two hours for both trials one and two. Least significant means are shown plus and minus the standard errors.

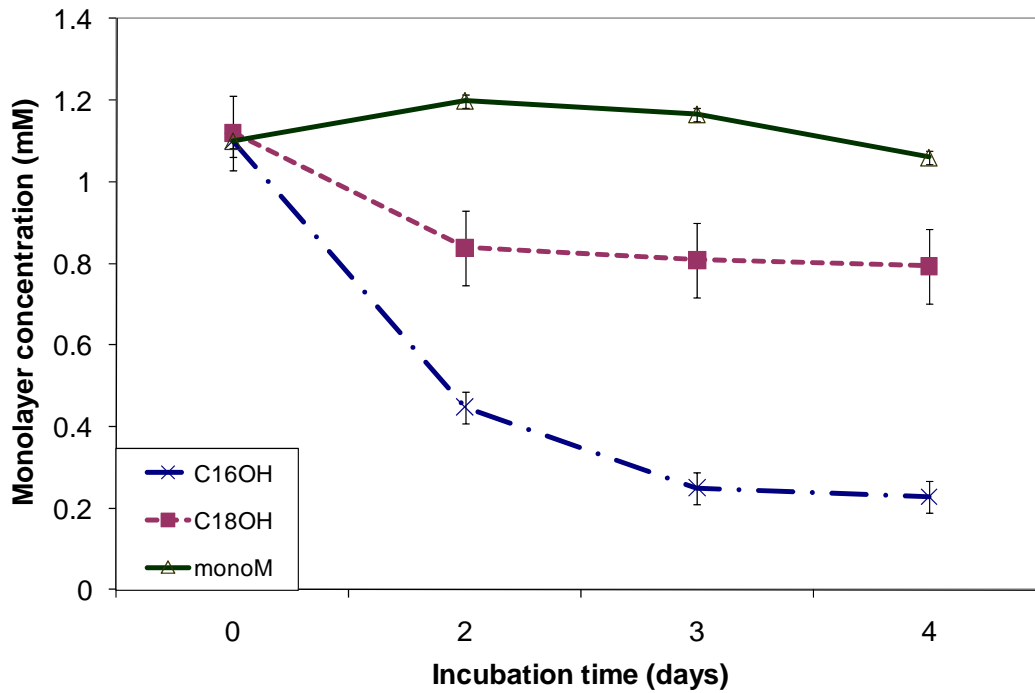


Figure 9. The concentration of the three monolayer compounds C16OH, C18OH and monoM, remaining in the mineral salts medium inoculated with *Acinetobacter* and incubated for up to four days.

Note: Results for trial two only are shown. The uninoculated controls of all three compounds were incubated for only two hours before extracting the monolayer compounds. Least significant means are shown plus and minus the standard errors.

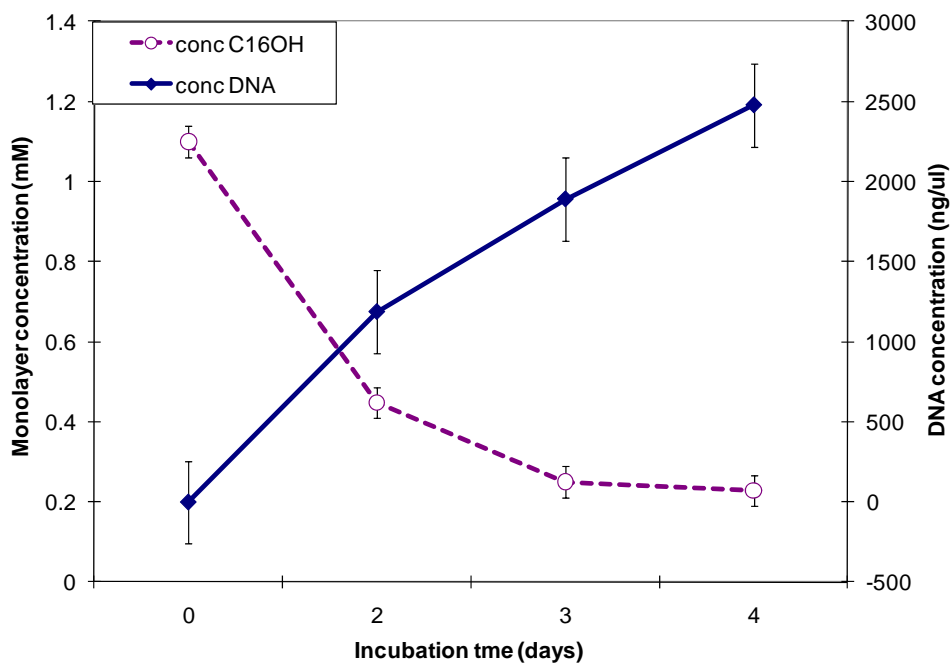


Figure 10. Concentrations of C16OH and microbial DNA in the mineral salts medium inoculated with *Acinetobacter* incubated for up to four days.

Note: Results for trial two only are shown. The concentration of C16OH in the uninoculated control has been adjusted to the recovery expected with four hours incubation. Least significant means are shown plus and minus the standard errors.

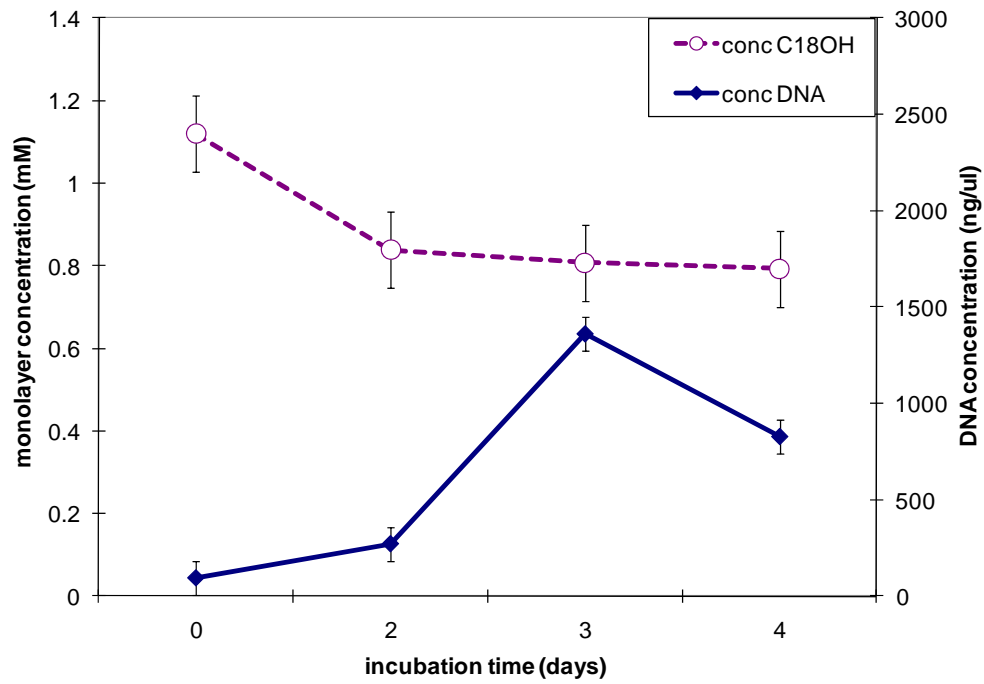


Figure 11. Concentrations of C18OH and microbial DNA in the mineral salts medium inoculated with Acinetobacter incubated for up to four days.

Note: Results for trial two only are shown. The concentration of C18OH in the uninoculated control has been adjusted to the recovery expected with four hours incubation. Least significant means are shown plus and minus the standard errors.

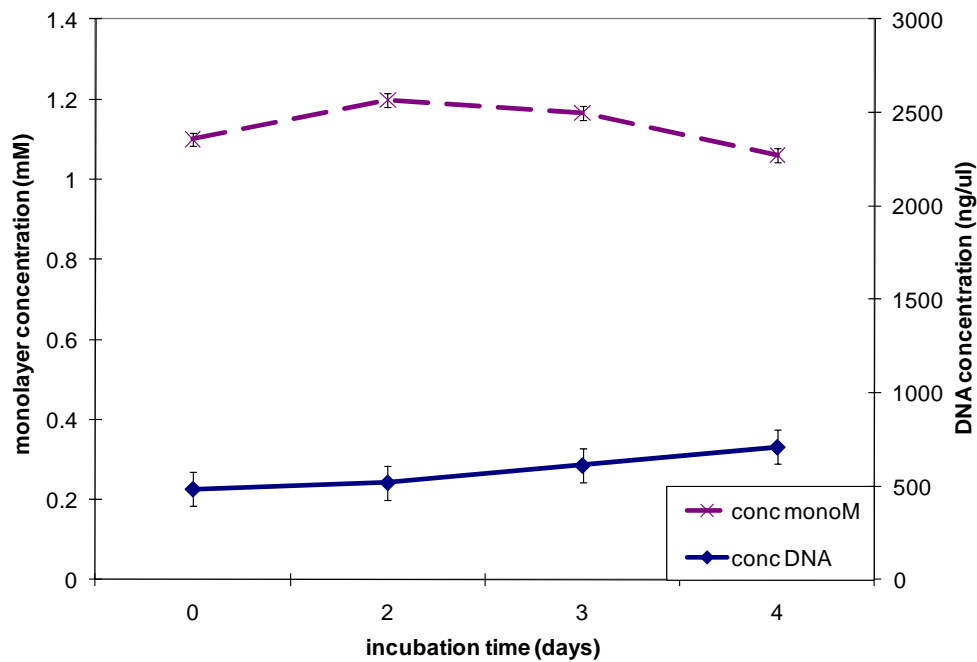


Figure 12. Concentrations of monoM and microbial DNA in the mineral salts medium inoculated with Acinetobacter incubated for up to four days.

Note: Results for trial two only are shown. The concentration of monoM in the uninoculated control has been adjusted to the recovery expected with four hours incubation. Least significant means are shown plus and minus the standard errors.

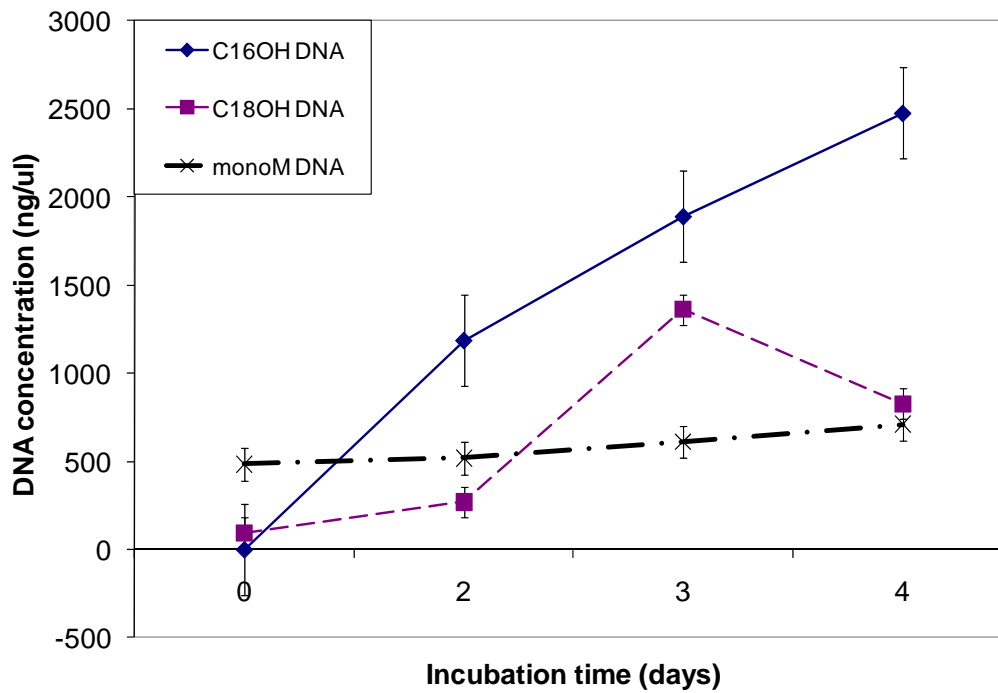


Figure 13. Microbial DNA extracted from three monolayer mineral salts media inoculated with *Acinetobacter*, incubated for up to four days.

Note: The monolayers C16OH, C18OH and monoM at a concentration of 1 mM were added to the broth medium as the only source of organic carbon available for microbial growth. Least significant means are shown plus and minus the standard errors.

Comparing the DNA concentrations for the three monolayers (Figure 13), all three compounds are used by the microbes to some extent within the four days of incubation. Hexadecanol degrades most easily, with the concentration of DNA increasing steadily over the four days (analysis of variance $P < 0.001$). Microbial use of octadecanol is slower (analysis of variance $P < 0.001$), reaching a peak by day 3. After day 3 the concentration of DNA drops, indicating that all of the readily available organic carbon has been exhausted and the cells have entered the stationary phase. As the microbial population enters the stationary phase, some microbial cells lyse, denaturing their DNA. This explains the drop in DNA concentration from day 3 to Day 4. In contrast to the other two monolayer compounds, the DNA content of the uninoculated monoM controls is not zero (Figure 13). The uninoculated controls start with a concentration of 484 ng/uL, with only a marginal, but statistically insignificant, increase to 710 ng/uL by day 4 ($P = 0.334$).

This result is unexpected as all of the uninoculated controls should register as zero. The results may reflect the order in which the samples were prepared. The ratio of the absorbance of the samples at wavelengths of 260 and 280 nm is used to assess the purity of the DNA sample. Ratios of less than 1.8 are considered contaminated, with either protein or phenol.

Results for samples incubated for two and three days were analysed first, with the controls and four days of incubation analysed last. The monoM DNA concentration for days two, three and four is uniformly low, as is the ratio of the absorbance values for all samples taken at wavelengths of 260 and 280 nm (values range from 1.286 to 2.136, with most registering below 1.6). By contrast, the ratio for the hexadecanol DNA results is consistently above 1.8, with most registering above 1.9. The ratio for the octadecanol samples is not as consistently high as for hexadecanol, but is consistently above 1.6. Because of the very low concentration of DNA present in the monoM samples, it is highly likely that the Nanospectrophotometer is detecting phenol and not DNA. While the spectrophotometer was being operated, consistent low readings affected the sensitivity of the instrument and may explain the results observed for the monoM control (uninoculated) samples.

4. Discussion

4.1. Water quality of Southeast Queensland Water Bodies

The range of water quality attributes recorded in this study (Table 2) are at the high end of the median values recorded for surface water in the Condamine-Balonne-Culgoa catchment (Kenway, 1993). Conductivity for the catchment study ranged from 96 to 1525 $\mu\text{S}/\text{cm}$. Readings for Lake Dyer, Cooby Dam, the Brimblecombe ring tank and the AgPlot tank are higher than this.

The AgPlot tank performed poorly in preliminary studies on the impact of poor water quality on the performance of current evaporation mitigation surface films (Morrison, 2007). The high conductivity in the tank (8585 $\mu\text{S}/\text{cm}$) was due to too much sodium hypochlorite being applied. Using saline bore water for irrigation from the Brimblecombe ring tank raised the conductivity of the water to 5910 $\mu\text{S}/\text{cm}$, and natural salt plumes in the Toowoomba region (Kenway, 1993) are most likely responsible for the high conductivity of Cooby Dam water (2467 $\mu\text{S}/\text{cm}$).

Agricultural crops such as cotton and beetroot are tolerant of salinity. Therefore, irrigation water storages may have a high conductivity where saline groundwater is used to supplement surface water supplies. The ions responsible for conductivity may adversely affect the performance of monolayer compounds. Changes in pH and electrolyte concentration are known to affect the stability of insoluble monolayers of low molecular mass (Binks, 1991).

The pH range for surface water resources in the Condamine-Balonne-Culgoa catchment was 7.0 to 8.1 (Kenway, 1993). The pH of the water bodies monitored in this study is more alkaline (Table 2), ranging from 7.8 for the Alderton ring tank to 9.1 for the AgPlot tank. The increase in pH in surface and groundwater is associated with salts such as calcium carbonate, which may also adversely affect the performance of monolayer compounds. Small-scale Langmuir trough studies are needed on some of the water bodies at the higher and lower ends of the water quality spectrum to establish how different water quality criteria affect the rate of spreading and compression of monolayer compounds, and their ability to suppress evaporative loss.

4.2. Microlayers and their Potential to Adversely Affect Monolayers

Microlayer enrichment was evident for all six of the water storages studied (Table 6). Of the water quality parameters studied, enrichment was greatest for the permanganate index, BOD, and UV absorbance (253.7 nm). As expected, results for the

permanganate index were positively correlated with UV absorbance (Pearson correlation 0.597, significant at 0.05 level, Table 5), but only marginally significant. The correlation between UV absorbance and the permanganate index calculated for a northern European study of six brown water lakes was also low (Pearson correlation 0.532, not significant at 0.05 level, data from Hessen 1985), but the correlation between bacterial biomass and UV was very high (Pearson correlation 0.74, significant at 0.001 level). In the current study, the Pearson correlation for UV and BOD (selected as an index of microbial activity) is very low (0.146) and not statistically significant ($P = 0.603$, $n = 15$).

The low level of significance may be due to the reactivity of microbial cells with permanganate. KMnO_4 concentrations of less than 10 mg/L oxidise microbial cells (Peterson *et al* 1995), which do not absorb light in the UV region. Humic materials derived from the decomposition of plants often are brown and readily absorb light in the ultraviolet range (Mrkva, 1983; Knuutinen *et al.*, 1988). The glycol groups and double bonds of lignified organic compounds are preferentially oxidised by KMnO_4 (Tirol-Padre & Ladha, 2004), explaining why UV absorbance is positively correlated with the permanganate index. Absorbance at 253.7 nm has been used as an alternative to the permanganate index for measuring the chemical oxygen demand of the water in water bodies containing high concentrations of old, humified material derived from sphagnum peat that does not support high levels of microbial activity (Mrkva, 1983)

In Australia, where fresh inputs of lignified organic compounds may support higher levels of microbial activity, both the permanganate index and UV absorbance will need to be measured to fully characterise microlayer and subsurface water samples. The changes recorded for the BOD, permanganate index and UV absorbance from the 2007 and 2008 sampling, indicate that monitoring over different seasonal conditions is also necessary to fully characterise the microlayers naturally present on water storages.

The difference in the microbial availability of humified materials in the microlayer is evident in the results for the Alderton Ring tank and Narda Lagoon (Table 4). Both water bodies have a very high permanganate COD (53.4 and 25.4 mg/L for Alderton and Narda Lagoon respectively), but only Narda Lagoon has a correspondingly high BOD (1.5 and 10.3 mg/L respectively). Studies on bacterial use of high molecular weight, dissolved organic carbon in humic lakes indicate that bacteria use the higher molecular weight compounds more readily than lower molecular weight compounds (Amon & Benner, 1996). The reduced microbial activity in the Alderton water samples

may reflect the fact that the humified material is absorbed onto bentonite clay particles not available for microbial degradation.

In clear Northern European lakes, high molecular weight compounds are less concentrated than in humic lakes, and the availability of the compounds for bacterial degradation differs (Tranvik, 1990). The author concluded the reason for this is that the quality of dissolved organic carbon in humic and clear lakes is different. In clear lakes, the complex nature of humic-like compounds limits their rate of decomposition to about 2 to 5 per cent per year (Xia et al., 2005). These humified substances do not contribute greatly to the microbially mediated BOD, but react readily with strong chemical oxidants such as potassium permanganate (COD). This difference in the age and quality of the dissolved organic carbon in a water storage and microbial activity may explain the lack of correlation between the permanganate index and the BOD (Table 5 and Figures 2 and 3).

The age of dissolved organic carbon in the microlayer is also likely to vary with seasonal conditions. The permanganate index and BOD readings for the subsurface water in Narda Lagoon sampled in 2007, a drought year when the water level was very low, were low relative to the microlayer samples (Table 7). However, after heavy summer rains had filled the lagoon to capacity in early 2008, the permanganate index and the BOD of the subsurface water had increased substantially, reducing the enrichment factor (Table 8).

The main source of humified organic compounds in the microlayer of lakes in the northern hemisphere is sphagnum peat (Hillbricht-Ilkowska & Kostrzewska-Szlakowska, 2004). In Australia, the high content of waxes and lignin in bark and leaves shed by the vegetation and litter fall occurring at the same time as seasonally dry periods (Bunn, 1986) may explain why the permanganate index and UV absorption for Australian water bodies is as high or higher than records for European humic lakes (Figures 4 and 5). The concentration of these photochemically and microbiologically reactive compounds in Australian water bodies may reduce the efficacy of artificial monolayer compounds applied to humic water storages. In contrast, water bodies in catchments that do not have high concentrations of woody native vegetation may have very low levels of microbial and photochemical activity and may respond better to the application of artificial monolayer compounds.

The water quality results of this study suggest that new monolayer compounds may need to be developed to cover the range of microbial and photochemical activity and

water quality conditions that occur in Australian water storages. A product that performs well on one water storage may perform poorly on another. Selecting the right product for a given water storage will be a key criterion for the technology to be economically attractive for irrigators and other water managers.

4.3. The Potential of Artificial Surface Films to Adversely Affect Aquatic Ecosystems

The existence of natural microlayers on the surface of water storages provides an ecological benchmark for assessing the impact of artificial surface films applied to water storages to control evaporative loss. Surface films increase surface temperature and decrease the rate of gas exchange by dampening capillary wave action (Gladyshev, 2002). Not all surface films suppress evaporative loss, but those that do must generate a high surface pressure (Barnes, 2008). Data from the water storages monitored in this study (Table 6) indicate that the surface pressure of the microlayers was way below the surface pressures recorded for monolayer compounds known to reduce evaporative loss (39 and 35 mN m^{-1} for hexadecanol and octadecanol respectively, Barnes 2005). However, the effect of wave dampening and changes to gas exchange and temperature are induced by surface films that have low surface pressures and do not reduce evaporative loss (Saylor et al., 2000). Therefore, the presence of a natural microlayer on a water body will also affect these processes.

The effect of surface films on air/water exchange varies with both the thickness and the chemical composition of the film. Increasing the thickness of a petroleum film from 2.5 to 40-50 nm decreased the ratio of the oxygen transfer coefficient on clean versus film water by a factor of 2.5 (Gladyshev, 2002). By contrast, an artificial monolayer mixture of octadecanol and hexadecanol decreased the oxygen transfer coefficient from 0.64 cm/h to 0.43 cm/h, a ratio reduction of only 1.4. By definition monolayers are one molecule thick, much thinner than multilayered surface films of petroleum or silicone oil. Microlayers are also much thinner than oil films, between 1 and 10 nm (Norkrans, 1980).

The composition of the monolayer will also affect its biochemical properties. Primary aliphatic alcohols with chain lengths from C_9 to C_{12} act as nonionic surfactants that kill micro-organisms and aquatic insect larvae by disrupting their membranes (Kubo et al., 2003; Hammond & Kubo, 1999). The anaesthetic effect responsible for the insecticidal properties of mid-chain length alkanols disappears as the carbon chain length increases from C_{11} to C_{15} . Monolayer compounds capable of suppressing evaporative loss have carbon chain lengths of C_{16} and above (Barnes, 2005).

These results indicate that monolayer compounds applied to water storages to reduce evaporative loss should have a negligible effect on aquatic ecology. The most extensive examination of the effect of monolayers on water quality was undertaken by Wixson (1966), using a mixture of the compounds hexadecanol and octadecanol. No reference was made to any pre-existing microlayer on the lake. The study was conducted in North America over a period of thirty days. Applying the monolayer to the clean water surface reduced oxygen diffusion by 10 to 15 per cent. However, this effect varied with the timing of measurements and over the duration of the trial. The author was concerned that the decrease in surface tension that occurred after applying the monolayer caused some filamentous algae to sink. Numbers of non-filamentous algae appeared to decrease over the first 15 days, but recovered to population densities above the control by the end of the trial. Bacterial populations increased over the duration of the trial as they used the monolayer compounds as a substrate. No significant effects of the monolayer on the fish species *Gambusia affinis* and *Fundulus notatus* were observed.

In the current study, applying hexadecanol in a hydrated lime carrier resulted in a substantial increase in the pH of the microlayer sample (Table 9). The potable tap water used in the study had a pH of 7.9 to 8.2. Surface water recording a pH of above 8 is considered alkaline for the Condamine-Balonne-Culgoa catchment (Kenway, 1993). Increasing the pH to 10.9 is highly likely to affect aquatic microbes and larger organisms that breathe and feed at the surface interface. The elevated pH reduced over time and did not appear to affect the activity of heterotrophic microlayer bacteria responsible for the BOD. The BOD for the hexadecanol-hydrated lime treatment in trial 1 was five to six times greater than for the clean water sample, and 60 per cent greater than the BOD recorded for the monoM monolayer formulation, which did not contain hydrated lime (Table 9). The BOD results for the silicone surface film suggest that the oil is scavenging oxygen. Under the conditions of the BOD test, the stopper on the Wheaton bottle excluded all air from the water sample. Applied to a water surface, open to the air interface, this ability to scavenge oxygen may not necessarily deplete the dissolved oxygen in the microlayer and the immediate subsurface water. Further study is required to determine whether including hydrated lime in the monolayer formulation or the oxygen scavenging properties of the silicone oil adversely affects aquatic organisms active at the air/water interface.

4.3.1. Microbial Degradation of Artificial Monolayer Compounds

The hydrophobicity of monolayer compounds provided a challenge for developing research methods. As a monolayer, the compounds had to be deposited reproducibly at the water interface. To achieve this, the compounds were dissolved in acetone and

placed on an orbital shaker to coat the internal surfaces of a 250 mL conical flask as the solvent evaporated. Ideally, when 20 mL of sterile MS broth is added, the amphiphiles should float to the surface and form a monolayer. The results for the control flasks used in the microbial degradation trials one and two indicate that the compounds did not detach as readily from the surface of the flasks as expected. Recovery of the monolayer compounds in the uninoculated control flasks was lower for trial two, where the flasks were incubated on a rotary shaker for two instead of the four hours used in trial one. This is reflected in Figures 6, 7 and 8 with the concentration of the monolayer compounds in the control flasks for trial one being up to 26 per cent less than for the controls. Despite a magnetic stirrer being used to produce a vortex for maximizing the mixing of the chloroform and water phases, some monolayer remained attached to the glass surface of flasks incubated for less than four hours.

Strains of the genus *Acinetobacter* can produce a diversity of enzymes to degrade aromatic compounds such as phenols, cresols, toluene, polyethylene glycol, and cyclohexane (Towner, 1992). Most other bacterial strains can only degrade such resilient compounds when present in mixed cultures, acting as a microbial consortium (Ghazali et al., 2004). Long-chain fatty alcohols such as hexadecanol are more readily degraded, given their similarity to lipid storage compounds used by many bacterial species (Uthoff et al., 2005). In water bodies with well-developed microlayers, the population of microbial species that can use hydrophobic saturated and unsaturated lipids such as the 16 carbon palmitic acid are likely to predominate (Norkrans, 1980). To attack both naturally produced and anthropogenic amphiphiles, bacteria secrete surfactants to improve interfacial contact of the living cells with the insoluble hydrocarbon (Bouchez-Naitali et al., 1999). Both *Pseudomonas* and *Acinetobacter* produce biosurfactants that reduce both the interfacial and surface tension of aqueous solutions in the process.

The production of biosurfactant by the *Acinetobacter* strains used in the microbial degradation studies in this report was evident as a viscose emulsion that collected at the chloroform-water interface in the separation funnels. The biosurfactant was also evident as a surface film coating the interior of the separation funnels once the chloroform and aqueous phases had been decanted. Initially, the microbial cells degrading the monolayer compounds were counted using the optical density of the cells in colloidal suspension when a sample of the culture was placed in a spectrophotometer set at a wavelength of 600 nm (Collins et al., 1989). This method was abandoned as the biosurfactant produced by the microbes emulsified both the monolayer compound and microbial cells. Instead of producing repeatable OD600

absorbance readings and consistent dilution plate counts, the biosurfactant caused the cells to clump. From a practical perspective, producing biosurfactants by aquatic microbes may interfere with artificial monolayers by emulsifying monolayer domains, disrupting the orderly packing necessary for the retardation of evaporative loss.

In the Introduction (Section 1.3), naturally occurring microlayer compounds were considered to have the potential to adversely affect artificial monolayers in three ways:

- As microbial substrates favouring the growth of microbes pre-adapted to degrade long-chain amphiphiles such as artificial monolayer compounds;
- As non-homologous amphiphilic hydrocarbons disrupting the orderly packing and/or forming holes in artificial monolayer films; and
- As chemically reactive humus-like compounds increasing photodegradation.

The results from this study suggest that a fourth adverse impact must be considered, and that is the existence of biosurfactants on water bodies at the time of application of artificial monolayer compounds.

In waters that have a well-developed microlayer, biosurfactants are highly likely to have been produced by microbes using these natural amphiphiles as organic substrates. Biosurfactants produced by bacteria such as *Acinetobacter* form very stable emulsions or dispersions and are effective at relatively low concentrations in aqueous solutions as all of the polymer partitions at the surface (Rosenberg & Ron, 1998). Their presence at the air-water interface may affect the packing of artificial monolayers applied to reduce evaporative loss, affecting the performance of the monolayer (refer 1.1.1). Testing candidate monolayer compounds for evaporative retardation and re-spreading in the presence of biosurfactants, should be included as a key performance criterion for screening the commercial feasibility of new compounds.

Acinetobacter strains can not only degrade a range of aromatic compounds, but also produce different biosurfactants. These properties are particularly relevant when selecting a microbial species for bioassays testing the resilience of different chemical compounds to microbial attack. Results for the recovery of the monolayer compounds and genomic DNA from the microbial broths indicate that the 16-chain compound hexadecanol degrades much more readily than the C18 compound octadecanol. The shape of the microbial DNA curve for octadecanol has a peak after three days incubation, dropping to a lower level by four days. This is consistent with microbial

growth in the stationary phase that occurs after the readily available organic carbon substrate in the medium has been exhausted (Madigan et al., 2003).

5. Conclusion

The results of this study suggest that by increasing the hydrophobic chain length by two carbons (from C16 to C18), the resilience of fatty alcohol monolayer compounds to microbial degradation can be increased. The increase in chain length may also reduce the rate at which the monolayer sublimates after it is applied to the water surface (Barnes, 2008). Unfortunately, the most microbially labile of the candidate compounds, hexadecanol, has been studied more intensively than the other more resilient compounds. The poor performance of hexadecanol, which has limited the commercial uptake of monolayer technology, can now in part be explained. By moving away from the C16 fatty alcohol, the resilience of artificial monolayers can be increased, improving the commercial feasibility of monolayer technology.

The scope of this study was restricted to assessing water quality parameters that may affect the performance of artificial monolayers. A key outcome is evidence that artificial monolayers based on long-chain fatty alcohols share many of the biochemical characteristics of naturally occurring microlayers (Section 4.3). Benchmarking the attributes of artificial monolayers against the attributes of Australian microlayers provides circumstantial evidence that the impact of artificial monolayers on the ecology of natural aquatic systems should be minimal. Investigating this further was beyond the scope of this project, however, the technical challenges encountered during the development of the microbial screen will be relevant to any future study of the toxicity of monolayers. The low water solubility of the monolayer compounds is a particular challenge, and must be considered in any future biological indicator assays (e.g. Ma & Chen, 2005). Toxicity testing needs to focus on ecological processes that occur within the microlayer or at the air/water interface. Testing that is restricted to monitoring impacts on subsurface water quality may produce very different outcomes to testing undertaken on microlayer samples.

The results of this study indicate that the concentration of humic materials in Australian brown water storages is higher than for humic water bodies in northern Europe and North America. The presence of an enriched microlayer with a high permanganate index is likely to enhance photodegradation. Hydrophobic pollutants such as oil and pesticides also accumulate in the microlayer (Larsen et al., 1974). Oxidative degradation is a property of microlayers responsible for the natural 'cleansing' of polluting hydrocarbons from the surface of lakes (Baier, 1970). Enriching the humified content of the microlayer may enhance photo- and microbial degradation, speeding up the process of bioremediation (Bertillon & Allard, 1996).

The humic-like organic compounds responsible for photodegradation are byproducts of the microbial use of complex, plant-derived organics such as lignins and lipids (Amine-Khodja et al., 2006; Bollag et al., 1998). This issue is relevant to organisations such as the CRC for Cotton Communities and Catchments, and the Condamine Alliance. Vegetating water storages such as ring tanks and tail drains may increase the permanganate index of the microlayer, enhancing the rate of photodegradation of pesticides. More work to establish a link between vegetated riparian zones and the rate of degradation of pesticides could be undertaken to contribute to industry best management practices for environmental management.

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