

# **Properties of Natural Microlayers on Australian Freshwater Storages and their Potential to Interact with Artificial Monolayers.**

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## *Abstract*

Microlayers are natural surface films derived from hydrophobic organic compounds that form on most lakes and streams. Holoarctic brown water lakes have been most commonly studied, with Australian research limited to marine microlayers. Artificial monolayers based on long-chain fatty alcohols have been applied to freshwater storages to reduce evaporative loss. As a water conservation strategy, monolayer technology was not widely adopted due to variable field performance. However, the role of natural microlayers in reducing monolayer performance has not previously been investigated.

In this study, microlayer and subsurface samples from six water storages in Queensland were characterized for water quality indices including biochemical oxygen demand, permanganate index and ultraviolet light absorbance. Microlayer enrichment in southeast Queensland is comparable to or higher than holoarctic lakes. Results indicate that microlayer compounds have the potential to disrupt monolayers in at least three ways: As substrates for microbes capable of degrading monolayer compounds, as chromophores accelerating photodegradation, and as impurities disrupting the molecular packing required to reduce evaporative loss. The knowledge gained from studying natural microlayers can also be used to benchmark novel monolayer compounds, to minimize their environmental impact on freshwater ecosystems.

**Key words:** BOD, UV absorbance, permanganate index

**Running head:** Impact of freshwater microlayers on monolayers

## Introduction

Water managers are increasingly considering the application of artificial surface films as a water conservation strategy, to minimize evaporative loss in open storages depleted by drought (Barnes 2008). Freshwater ecologists have expressed concern about this practice, as the physical properties of surface films affect the temperature, gaseous diffusion, surface tension and other ecologically important processes that occur at the air-water interface (eg. Parker and Barsom 1970). Marine surface films have been studied most intensively, originally to investigate the adverse impact of oil spills on marine ecology (Norkrans 1980). The most significant outcome of these early studies was the realization that even in unpolluted water, a variety of naturally amphiphilic substances (compounds containing a hydrophilic head and a hydrophobic tail) accumulate at the air-water interface to produce a surface film or microlayer (Marshall 1976). The maximum depth of natural microlayers varies from a single molecular monolayer to a thickness of several millimeters.

Naturally derived marine microlayers are formed from lipids including free and combined fatty acids, fatty alcohols and hydrocarbons, synthesized predominantly by marine phytoplankton (Meyers and Kawka 1982). Microlayers formed on brown water lakes in the holarctic region have been the most commonly studied freshwater surface films. The amphiphilic, humic substances are derived mainly from sphagnum peat (Hillbrecht-Ilkowska and Kostrzewska-Szlakowska 2004, Hongve 1999), and serve as substrate for secondary bacterial production (Hessen *et al.* 1997). The concentration of humic compounds in microlayers can increase microbial activity 10 to over 100 times above activity in subsurface water (Norkrans 1980, Munster *et al.* 1998). Australia also has brown-water lakes, but most studies have focused on coloured, dissolved organic matter referred to as gilvin, in subsurface water samples (Wrigley and Cowan 1995, Song Qiu *et al.* 2005). In the Australian studies, the humic compounds originate predominantly from *Eucalyptus* leaf and bark litter leachate. There appear to be no published studies on the properties of natural microlayers on Australian freshwater storages.

The physical impact of naturally occurring microlayers at the air-water interface is very similar to the impact of artificial monolayers (Norkrans 1980, Gladyshev 2002). During the 1950s and 1960s artificial monolayers based on the fatty alcohols hexadecanol and octadecanol were extensively studied for their potential to reduce evaporative loss, as a water

conservation strategy (Frenkiel 1965, Barnes 2008). Long-chain fatty alcohols were most commonly selected due to the ability of the hydrophobic carbon chains to pack tightly together to resist evaporative loss, the small quantity required to cover a large surface area, and their very low toxicity to aquatic life (Wixson 1966). However, the technology was not widely adopted as field trials yielded variable results, and the compounds had to be re-applied every two to four days (Barnes 2008).

The microbial enrichment within natural microlayers on water bodies may in part explain the variable performance of artificial monolayers in the field. Laboratory studies confirm aquatic bacteria readily degrade hexadecanol (Chang *et al.* 1962). Humified microlayer compounds may also physically degrade artificial monolayers by disrupting the ordered molecular packing required to reduce evaporative loss (Norkrans 1980). They may also enhance the rate of photodegradation of artificial monolayer compounds as humified organic compounds strongly absorb ultraviolet light (Bertilsson and Allard 1996). Photochemically generated singlet oxygen and hydroxyl and alkylperoxy radicals are known to degrade compounds such as 2,4-D esters and 2-chlorophenol directly, and indirectly by transferring energy from excited humic compounds to the pollutants (Kawaguchi 1992). In the present study, the potential for microlayers to adversely affect the performance of artificial fatty alcohol monolayers was investigated by comparing the properties of microlayer and subsurface water samples collected from six open water storages in southeast Queensland.

## Materials and Methods

### *Site and Water Sampling Descriptions*

Six water bodies located in southeast Queensland were sampled during November and December 2007, towards the end of an extended period of drought. One of the water bodies was sampled again after rain had replenished water levels, in July 2008. The water bodies were selected on the type of riparian vegetation within the immediate catchment (cleared agricultural land or woody remnant native vegetation), and the main source of the water (overland flow, bore water, or pumped from a water course during peak flow). Sampling occurred at dawn or dusk, when the wind velocity was least.

A microlayer plate sampler was constructed from 10 mm thick hydrophobic Teflon sheet (Larsson *et al.* 1974). The dimensions of the finished plate were 20.5 cm long by 20.0 cm wide. Conical indents were drilled into the sheet, perforating the base of the plate with small holes 1 mm in diameter. The microlayer was sampled by firmly placing the plate into the surface of the water, to a depth just less than the thickness of the plate. The plate was removed vertically from the surface, momentarily allowing subsurface water to drain, before the microlayer sample held within the conical indents was collected by shaking the plate above a Teflon coated baking tray (Hillbrecht-Ilkowska and Kostrzewska-Szlakowska 2004). Prior to sampling, all equipment was rinsed with 96% methanol to remove all lipid and microbial contaminants and air-dried (Kostrzewska-Szlakowska 2005). The plate was dipped repeatedly into minimally disturbed sections of the water surface until a volume of 800 mL had been collected. A composite subsurface sample of 800 ml was collected from a depth of 5-10 mm below the surface using a glass bottle (Hatcher and 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. The surface pressure of the microlayer on the windward and lee shores of two water storages was measured by applying a series of indicator oils to the surface dropwise, until the concentration of the indicator oil was equivalent to the spreading pressure of the microlayer (Barnes 2008). The indicator oils were prepared by varying the concentration of dodecanol in mineral oil to produce a range of surface pressures from 6 to 35 mNm<sup>-1</sup>. The surface pressure of each of the indicator oils was calibrated by applying a drop of each concentration to deionised water in a Langmuir trough, using a Wilhelmy plate to measure the change in surface tension. The thickness of the microlayer sample removed by the Teflon plate was estimated by pouring the volume of water

adhering to the conical indentations of one plate sample collected in the Teflon tray, into one plastic vial. The volume of water collected from ten consecutive dips was used to calculate the mean and standard deviation sampling depth. The pre-weighed sampling vials were re-weighed, and the depth of sampling was calculated using the surface area of the plate, dipped into tap water contained in a plastic trough with a surface pressure of 6 mN/m.

#### *Characterisation of the Microlayer and Subsurface Water Samples*

Water samples were stored at 4<sup>0</sup>C in the dark prior to analysis. Electrical conductivity, pH and the five-day biochemical oxygen demand (BOD) were analysed within three days of sample collection, and the permanganate index, ultraviolet light absorbance and chloride ion concentration were analysed within 10 days of collection. 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 BOD was 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). Results were expressed as the permanganate index (mg of permanganate oxidized per L of water sample based on 1 mL of 0.002 M KMnO<sub>4</sub> corresponding to 0.316 mg KMnO<sub>4</sub>), or as the permanganate chemical oxygen demand (mg of oxygen consumed per L based on 1 mL of 0.002 M KMnO<sub>4</sub> corresponding to 0.08 mg O<sub>2</sub>). 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 prior to boiling, with the sulfuric acid added immediately before the addition of 15 mL of oxalic acid. The water samples were filtered through glass microfibre filter paper, prior to recording their absorbance at 253.7 nm (UV absorbance) 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 significance of microlayer enrichment was analysed using the Wilcoxon signed ranks test. The Pearson correlation coefficient was calculated to investigate the relationship between the chemical and biochemical parameters recorded for the water samples (Systat software package version 5). Data obtained for the permanganate chemical oxygen demand for the Australian water storages were compared with published data on

humic lakes sampled in Norway (Hessen 1985). The UV absorbance results recorded for the Australian water storages were compared with published data from Finnish lakes (Munster *et al.* 1998) and the enrichment factors were compared with published results from humic lakes in Poland (Kostrzewska-Szlakowska 2005).

### *Heterotrophic Bacterial Population Density*

Five water storages within the Lockyer Valley of southeast Queensland were sampled after summer rain in February and March 2009, to monitor the population density of heterotrophic bacteria as a function of the concentration of humified organic compounds in the water sample. The storages included Narda Lagoon and Lake Dyer, and three agricultural water storages filled during peak overland flow events (Logan's Storage, 16 ha) or during peak creek flow and supplemented with bore water (Caffey Dams, 2 ha). The surface pressure at the time of sampling was measured using indicator oils, and the concentration of humified compounds in microlayer and subsurface water samples was estimated by measuring the UV absorbance. The density of heterotrophic bacteria adapted for utilizing amphiphilic compounds in microlayer and immediate subsurface water samples was estimated using the Most Probable Number method (Collins *et al.* 1989). The indicator dye resazurin was added to a minimal salts broth (Bouchez *et al.* 1995), with phenol added as the sole organic carbon source at a concentration of 300 mg L<sup>-1</sup> (DiGeronimo *et al.* 1978). Preliminary identification of phenol-utilising bacteria was undertaken by subculturing positive samples of the resazurin phenol broth into a phenol minimal salts medium and streak-plating cloudy cultures onto soy tripticase agar. Colonies were putatively identified to genus using the result of the Gram's stain and colony and cell morphology (Collins *et al.* 1989). Where mixed cultures were evident, isolates were re-subcultured into the phenol minimal salts medium to confirm their ability to utilize phenol. The relationship between the concentration of humified compounds and the population of phenol-degrading bacteria was analysed using linear regression (Systat software package version 5).

## **Results**

### *Water storage characteristics*

The above-ground, lined water tank located in the Agricultural Plot at the University of Southern Queensland was included in the study, as trials on the efficacy of a commercial monolayer product based on hexadecanol indicated poor performance in reducing evaporative loss on this water storage (Table 1). The diameter of the tank was 10 m, filled to a depth of 0.8

m with local bore water. Duck activity over the duration of successive trials stimulated the growth of algae to levels that were much greater than that observed in the other water storages. The chloride concentration, conductivity and pH of the water were very high (Table 2), due to repeat applications of sodium hypochlorite attempting to control algal growth. The conductivity of water in the Forest Hill ring tank was also high as this storage was filled with saline bore water. The water storage that most closely resembled a humic lake was Narda Lagoon. The water was dark brown, derived from the phenolics and tannins leached from the *Acacia* and *Eucalyptus* trees in the riparian zone, and from hardwood sawdust produced at the sawmill located on the bank of the lagoon (Table 1). The water sampled from the Alderton ring tank was highly coloured and turbid, due to bentonite clay suspended in water pumped during peak flows from a creek bordered with remnant native vegetation. The water in all six storages had an alkaline pH, with the conductivity varying from 207  $\mu\text{Sm}^{-1}$  for the Alderton ring tank to 8585  $\mu\text{Sm}^{-1}$  for the Agricultural Plot tank (Table 2). Across all six water storages there was very little evidence of microlayer enrichment for pH ( $P = 0.310$ ) or conductivity ( $P = 0.050$ ).

Insert Tables 1 and 2 as close as possible

#### *Microlayer characteristics*

Visual evidence of a microlayer was most obvious at Narda Lagoon. Streaks of small particles formed in bands on the lee shore. Surface streaks were also observed on the lee shore of Cooby Dam and Lake Dyer, but not to the same extent as at Narda Lagoon. The high turbidity and wave action during sampling at Alderton made the visual detection of any surface film difficult. Preliminary results for Lake Dyer and Narda Lagoon indicated that the surface pressure of the microlayer was greatest close to the lee shoreline, reducing to  $\leq 6 \text{ mNm}^{-1}$  at a distance of one or two meters from the shore. This change in surface pressure affected the number of Teflon plate dips required to produce a microlayer volume of 800 mL. The higher the surface pressure, the more dips required to produce the sample volume (less surface tension). For a water surface with a surface pressure of 6 mN/m, the calculated microlayer sampling depth varied from 11 to 25 microns (mean 18.4  $\mu\text{m}$ , standard deviation 4.2  $\mu\text{m}$ ). Small changes in the shoreline such as the density of emergent vegetation or the presence of a headland or a bay also affected the surface pressure. The highest reading for Narda Lagoon was 35  $\text{mNm}^{-1}$  on the lee shore between clumps of floating water hyacinth, and the highest

reading for Lake Dyer was  $11.0 \text{ mNm}^{-1}$  on the lee shore between emergent reeds. To accommodate the spatial and temporal changes in surface pressure, a composite microlayer sample was collected from multiple sampling points within 2 m of the shoreline of each water storage, until the volume approximated 800 mL. On larger water storages, separate 800 mL composite samples were collected from the lee and windward shores.

The high algal growth evident in the Agricultural Plot tank was reflected in the very high BOD values for the subsurface and microlayer water samples (7.7 and  $11.9 \text{ mgL}^{-1}$  respectively, Table 3). The BOD values were the highest recorded for all six water bodies, but the Mn index and UV absorbance values were lower than Narda Lagoon, Lake Dyer and the Alderton water storages. These three water bodies share the characteristics of having a wooded canopy in the catchment area (Table 1). Cooby Dam also has a wooded catchment, but the total volume of water is greater, effectively diluting the concentration of humic compounds in the water storage. The Narda Lagoon and Alderton storages contained the most highly coloured water, and had the highest Mn index (Table 3).

Insert Table 3 as close as possible

All water samples were pre-treated by filtering through glass fibre filter paper prior to measuring UV absorbance, chloride ion concentration and the Mn Index. UV absorbance for the Alderton water samples could not be tested as the fine bentonite particles passed through the glass fibre filter paper and interfered with light penetration. All water storages showed evidence of enrichment in the microlayer for the BOD ( $P = 0.015$ ), Mn index ( $P = 0.028$ ), chloride ion concentration ( $P = 0.011$ ), and UV absorbance ( $P = 0.012$ , Table 4). There was some evidence of greater enrichment on the lee shore relative to the windward shore, but as sampling was undertaken under calm conditions the shore designation may not represent the direction of the prevailing wind. The enrichment factors were greatest for Narda Lagoon, recording 9.8 for BOD, 2.3 for Mn Index and 1.3 for UV absorbance. Enrichment values for the Lake Dyer and Alderton storages were lower than Narda Lagoon, due to high readings in both the microlayer and subsurface water samples (Table 3). The Narda Lagoon enrichment factors dropped substantially after summer rains in 2008 had replenished the storage to full capacity. Overland flow substantially increased the concentration of humified organics in the subsurface water relative to the microlayer concentration (BOD 4.4 and 8.7, Mn Index 54.6 and 98.7, UV absorbance 1.10 and 1.04 respectively). Results for the Pearson Multiple

Correlation test combining subsurface and microlayer samples indicate that the permanganate index was positively correlated with UV absorbance (0.597 P= 0.019, n= 15), and negatively correlated with chloride (-0.584, P= 0.011, n= 18). The result for BOD and Mn index was negative and not significant (-0.29, P= 0.228, n= 19), and the result for UV and BOD was positive but not significant (0.146, P= 0.603, n= 15). The Mn index was expected to be positively correlated with BOD, as humified organic compounds are considered to be the main organic substrate available for fresh water heterotrophic microorganisms (Norkrans 1980, Munster *et al.* 1998). Across all water storages both the BOD and the permanganate COD readings were higher in the microlayer, but for the Alderton storage in particular, the BOD was substantially lower than the COD (Figure 1). Readings for the BOD most closely matched the COD for the water storages recording the lowest Mn index and UV absorbance (Forest Hill ring tank and Cooby Dam, Table 3).

Insert Table 4 and Figure 1 as close as possible

The range of UV absorbance readings recorded for subsurface and microlayer samples from southeast Queensland in 2007 (Table 4), are similar to those recorded for small Finnish boreal lakes (0.14 to 0.70, and 0.16 to 0.77 absorbance units, Munster *et al.* 1998). However, after heavy rain replenished the Narda Lagoon storage, the UV absorbance of the water samples increased to 1.10 and 1.04 absorbance units (subsurface and microlayer samples respectively). The enrichment factors for UV absorbance for the southeast Queensland storages sampled in 2007 were all very close to the highest value recorded for humic lakes in Poland (Figure 2), and close to or above the highest value of 1.1 calculated for Finnish lakes (Munster *et al.* 1998). The permanganate COD values were as high, or higher than those recorded for humic lakes in Norway (Figure 3).

Insert Figures 2 and 3 as close as practicable

#### *Heterotrophic bacterial population density*

With the exception of the Logan water storage, the population of phenol-degrading bacteria varied consistently with the UV absorbance reading of the water samples (Figure 4). The population of phenol-degrading bacteria was highest in the Narda Lagoon microlayer sample, which had the highest UV absorbance and a high surface pressure (35 mNm<sup>-1</sup>). The lowest populations were in the subsurface water samples of both Caffey dams, which had the lowest

concentration of humified organic compounds and the lowest surface pressure ( $< 6 \text{ mNm}^{-1}$ ). Bacterial populations were high at Logan's storage, despite having a low UV absorbance. The surface pressure of the Logan storage was also low ( $< 6 \text{ mNm}^{-1}$ ). Cream, non-motile colonies of Gram negative, short rods putatively identified as *Acinetobacter* were isolated from four of the five water samples (Narda Lagoon, Lake Dyer, Caffey lower dam, Logan's storage), whereas cream-yellow, motile colonies of Gram negative long rods putatively identified as *Pseudomonas* were isolated from Narda Lagoon only. The regression of bacterial population density with UV absorbance was not statistically significant if the Logan storage results were included ( $P= 0.005$ ,  $R^2$  0.62,  $n= 10$ ). However, if the results for Logan's storage were excluded, the regression was significant ( $P= 0.0009$ ,  $R^2$  0.86,  $n= 9$ ). The regression equation for phenol-degrading bacterial populations as a function of UV absorbance excluding results from Logan's storage was:

$$\text{Bacterial density (colony forming units per 100 mL)} = 122.6 \times \text{UVabs} - 16.19$$

## Discussion

Visual and physical evidence of the existence of surface films on fresh water bodies was first recorded in 1949, on lakes in England (Goldacre 1949). Humic substances are predominantly responsible for the increase in the surface pressure of freshwater microlayers, inducing changes in the surface tension of water comparable to that of hydrophobic fatty surfactants (Cosovic and Vojvodic 1987). Pretreatment such as filtration, dilution and centrifugation does not affect the humic compounds in freshwater microlayer samples, indicating that they are more stable than marine humic compounds derived from phytoplankton. Microlayers were evident on all of the water storages sampled in this study (Table 4), but zones of relatively high surface pressure recorded on Narda Lagoon and Lake Dyer were restricted to within the shelter of floating or emergent vegetation, or within the shelter of a headland or bay. Surface pressures were uniformly low on open water (less than  $6 \text{ mNm}^{-1}$ ). Surface pressures above  $30 \text{ mNm}^{-1}$  are required consistently across a water surface, to effectively retard evaporative loss (Barnes 2008). Surface pressure is the reduction in the surface tension of water, a property which is critical for the survival of insects such as water striders and siphon-breathing mosquito and midge larvae, and pupae (Wiltzius 1967).

The high Mn indices recorded for all microlayer water samples (Table 4) in the absence of correspondingly high surface pressures indicate the presence of amphiphilic, humified compounds that may be too heterogeneous in chemical structure to produce the tight packing

required to uniformly increase surface pressure. Under laboratory conditions, monolayers of pure, long-chain fatty alcohols such as hexadecanol compress to  $10 \text{ mNm}^{-1}$  and greater, decreasing evaporative loss by 60 to 90% (Gladyshev 2002). However, the addition of short-chain alcohols or other amphiphilic impurities within the monolayer can substantially increase the loss of water vapour through ‘holes’ formed in the physical structure (Barnes 1997). The poor performance of the artificial monolayer applied to the Agricultural Plot water tank during field trials (unpublished data), may have been due to pre-existing microlayer compounds disrupting the monolayer structure.

The microlayer and subsurface water samples from the Agricultural Plot tank also had very high BOD readings (Table 4), indicating the potential to support high levels of bacterial activity. However, not all of the storages recording high Mn indices had correspondingly high BOD readings (Figure 1). For example, the Alderton samples had the highest Mn index (186.8 and  $210.8 \text{ mgL}^{-1}$  for subsurface and microlayer samples respectively), with the second lowest BOD readings ( $1.8$  and  $2.1 \text{ mgL}^{-1}$  respectively). In the case of the Alderton sample, this lack of correlation may be due to the fine clay particles adsorbing the humified compounds, rendering them less available for microbial metabolism. The affinity of kaolin clay minerals to adsorb waxy, organic compounds has been used by land managers to ameliorate water-repellant sands in Western Australia (McKissock *et al.* 2000). The lack of correlation between BOD and the Mn Index (Figure 1) may also be due to the age and complexity of the humified organic material. Older, more resilient humified material resists microbial attack, whereas newer material with a higher molecular mass is more readily degraded (Tranvik 1990, Xia *et al.* 2005). The Alderton storage was constructed within the last 5 years, indicating that the humified organics are relatively recent in origin. The organic material in the Agricultural Plot tank was even more recent, derived from algae and duck excreta after bore water was added to the tanks (Table 1). The organic material in Narda Lagoon was much more heterogeneous, with very resilient material potentially dating back as far as the 1930s when the sawmill was commissioned. Leaf and bark litter leachate produced from Australian native trees contains a higher concentration of lignin and phenolic compounds than leachate produced from European trees (Bunn 1986). This may explain the higher permanganate oxygen demand of microlayer water samples from southeast Queensland storages that have wooded riparian zones and/or water catchments (Table 1 and Figure 3). The timing of litter fall in Australia with seasonally dry conditions also concentrates humified material in water storages, with storm surges introducing fresh substrate. These dynamics suggest that open water storages in Australia may

provide more of a challenge for the deployment of artificial monolayer compounds as a water conservation strategy, than open water storages in the northern hemisphere.

The level of humification in water storages in southeast Queensland is as high, or higher than concentrations recorded for humic lakes in northern Europe (Figures 2 and 3). As chromophores, the presence of these compounds in the microlayer is likely to accelerate photodegradation (Kawaguchi 1992). Freshwater humic substances are comprised of carboxylic acids, alcohols, aromatic rings and aliphatic chains (Samios *et al.* 2007). Organic compounds with an aromatic ring structure absorb UV most strongly, with absorbance increasing as pH, total carbon content and molecular weight increase (Hautala *et al.* 2000). Permanganate is known to preferentially oxidize these compounds (Tirol-Padre and Ladha 2004). Studies from northern Europe indicate that the permanganate COD of lake water is more significantly correlated with UV absorbance than the dichromate COD (Mrkva 1983). The regression was positive and linear, with the correlation coefficient increasing as the level of organic pollution in the river system increased. In our study, the permanganate index of water samples collected from southeast Queensland was also positively correlated with UV absorbance (0.597,  $P=0.019$ ,  $n=15$ ), indicating that UV absorbance is a good indicator of the concentration of aromatic compounds in freshwater samples.

Humified, UV absorbing compounds have been identified as consistent components of marine microlayers (Carlson 1982). Carlson postulated that polymeric phenolic materials produced by macroalgae chemically interact with amino acids, carbohydrate or lipid compounds to form the amphiphilic complexes that characterise marine microlayer slicks. For the water storages sampled in 2009, UV absorbance was positively correlated with the population density of phenol-degrading bacteria for four of the five sites sampled (Figure 4). The site that did not show this trend (Logan's Storage) had very low levels of UV absorbance but very high population levels of phenol-degrading bacteria. Of the five sites sampled, this site was the only water body prone to algal blooms. Microalgae may also produce amphiphilic phenolic materials (Cosovic and Vojvodic 1987), providing an alternative substrate for microlayer bacteria in water bodies with low concentrations of phenolic materials derived from leaf and bark litter. To utilise amphiphilic compounds characteristic of both natural microlayers and artificial monolayers, bacteria must produce surfactants (Neu 1996). The phenol-degrading bacteria isolated in this study were putatively identified as *Acinetobacter* and *Pseudomonas*, genera containing species known to synthesise a range of biosurfactants. A key property of

biosurfactants is the capacity to reduce surface and interfacial tension at interfaces between liquids, solids and gases, effectively dispersing amphiphilic compounds such as natural microlayers and artificial monolayers as emulsions (Kitamoto *et al.* 2009).

In conclusion, the results from this trial indicate that the very high concentration of humified organic compounds present in the microlayer and subsurface water of open water storages in southeast Queensland (Table 3, Figures 2 and 3) is predominantly derived from the high phenolic content of the litter produced by Australian native trees present in the water catchment (Table 1). These humified microlayers have the potential to adversely interact with artificial monolayer compounds in at least three ways. As microbial substrates supporting the growth of microbes pre-adapted to degrade amphiphilic compounds such as hexadecanol; as amphiphilic surfactants physically disrupting the structure of monolayer films; and as chemically reactive aromatic chromophores increasing the rate of photodegradation. The development of new, improved monolayer compounds must address these challenges if the technology is to become commercially feasible for deployment on Australian water storages. The knowledge gained from studying natural microlayers can also be used to benchmark new monolayer compounds, to minimize their environmental impact. If the properties of new, improved monolayer compounds can be demonstrated as being ecologically equivalent to resilient, naturally occurring microlayer compounds, their potential to adversely affect fresh water ecosystems should be minimal.

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**Table 1: Locality, function and catchment characteristics of the six open water storages in Southeast Queensland, sampled for microlayer and subsurface water in October and November 2007.**

Name and Locality	Water source	Surface area at full capacity	Riparian Vegetation	Function
University Agricultural Plot water tank, Toowoomba (AgPl)	Reticulated town water, rainwater	0.008 ha	None, but high levels of algal growth	Experimental site for artificial monolayer testing
Narda Lagoon, Laidley (NL)	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 (LD)	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 (FH)	Peak flow off-take from Sandy Creek, and bore water	0.5 ha	Grassland and intensive cropping land	Farm irrigation storage
Cooby Dam, Meringandan (CD)	Cooby Creek, overland flow	306 ha	Eucalypt woodland on sodic subsoil	Municipal water supply for Toowoomba
Alderton ring tank, Yulabilla (Ald)	Peak flow off-take from Dogwood Creek	45 ha	Eucalypt woodland, bentonite clay subsoil	Recently constructed farm irrigation storage

**Table 2: Chemical water quality of the subsurface and microlayer water samples taken from six open water storages in Southeast Queensland. For large water storages, separate samples were taken from the lee (L) and windward (W) shores.**

	pH units		Electrical conductivity μS/m		Chloride ion concentration mg/L	
	subsurface	microlayer	subsurface	microlayer	subsurface	microlayer
Agricultural Plot	9.1	9.1	8240	8585	1569	1610
Narda Lagoon	8.4	8.2	260	280	33	37
Lake Dyer	8.1	8.2	1760	1720	572 W 571 L	570 W 574 L
Forest Hill	8.4	8.4	5855	5910	1789	1942
Cooby Dam,	8.9 W 8.7 L	8.8 W 8.8 L	2368 W 2417 L	2467 W 2501 L	676 W 681 L	691 W 701 L
Alderton	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

**Table 3: Biochemical water quality of the subsurface and microlayer water samples taken from six open water storages in Southeast Queensland. For large water storages, separate samples were taken from the lee (L) and windward (W) shores. UV readings for Alderton are not available (na) due to interference from suspended clay particles that passed through the glass fibre filter paper.**

	BOD5 O <sub>2</sub> consumed mg/L		Permanganate Index MnO <sub>4</sub> consumed mg/L		UV absorbance Absorbance units	
	subsurface	microlayer	subsurface	microlayer	subsurface	microlayer
	Agricultural Plot	7.7	11.9	42.6	83.8	0.27
Narda Lagoon	1.1W 1.3 L	10.3 L	43.2 W	94.2 W 100.3 L	0.35 W 0.36 L	0.47 W 0.45 L
Lake Dyer	4.8 W 5.2 L	6.2 W 6.4 L	53.9 W 53.7 L	48.2 W 56.9 L	0.55 W 0.58 L	0.61 W 0.61 L
Forest Hill	2.3	9.8	23.8	35.0	0.07	0.07
Cooby Dam,	2.2 W 2.4 L	3.5 W 5.3 L	25.9 W 27.1 L	31.0 W 31.6 L	0.12 W 0.13 L	0.15 W 0.14 L
Alderton	1.8 W 1.8 L	1.5 W 2.1 L	186.3 W 186.8 L	202.9 W 210.8 L	na	na

**Table 4: Enrichment factors, calculated as the ratio of the microlayer value divided by the subsurface value, of six water quality attributes recorded for the six open water storages located in Southeast Queensland. For large water storages, separate samples were taken from the lee (L) and windward (W) shores. Results for Narda Lagoon include a repeat sampling in May 2008, after heavy summer rains had replenished the storage.**

	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 2007	0.98	1.08	1.13	9.81	2.32 W 2.18 L	1.34 W 1.24 L
Narda Lagoon 2008				1.18 W 2.01 L	1.82 W 1.81 L	0.94 W 0.90 L
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
Forest Hill	1.00	1.01	1.09	4.20	1.47	1.05
Cooby Dam,	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	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

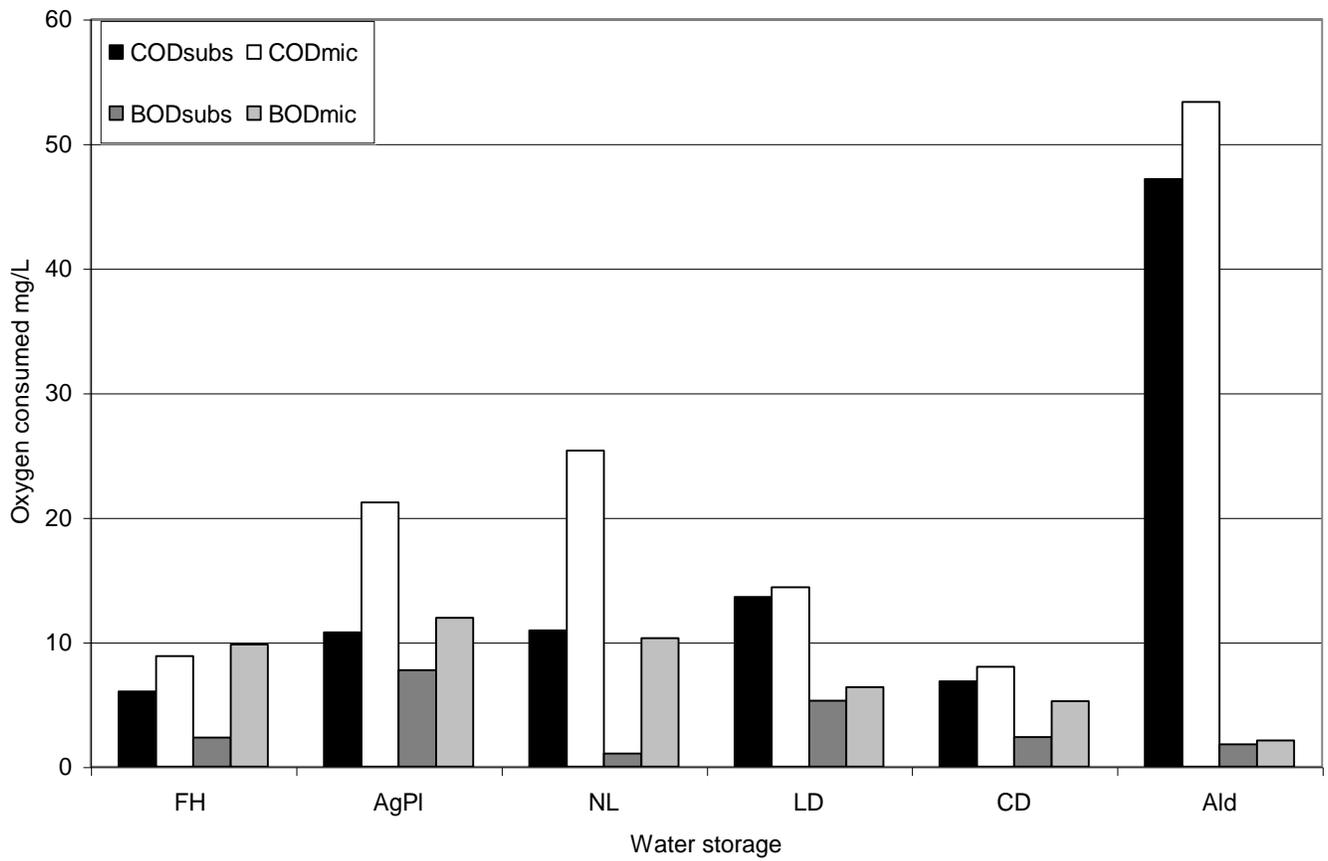
## Captions for Figures

Figure 1: A comparison of the permanganate chemical oxygen demand (COD) and the 5-day biochemical oxygen demand (BOD) for microlayer (mic) and subsurface (subs) water samples from six open storages located in southeast Queensland. Only the highest values have been provided for larger water storages where lee and windward shores were sampled for analysis. FH is Forest Hill, AgPl is the Agricultural Plot tank at the University of Southern Qld, NL is Narda Lagoon, LD is Lake Dyer, CD is Cooby Dam and Ald is the Alderton water storage.

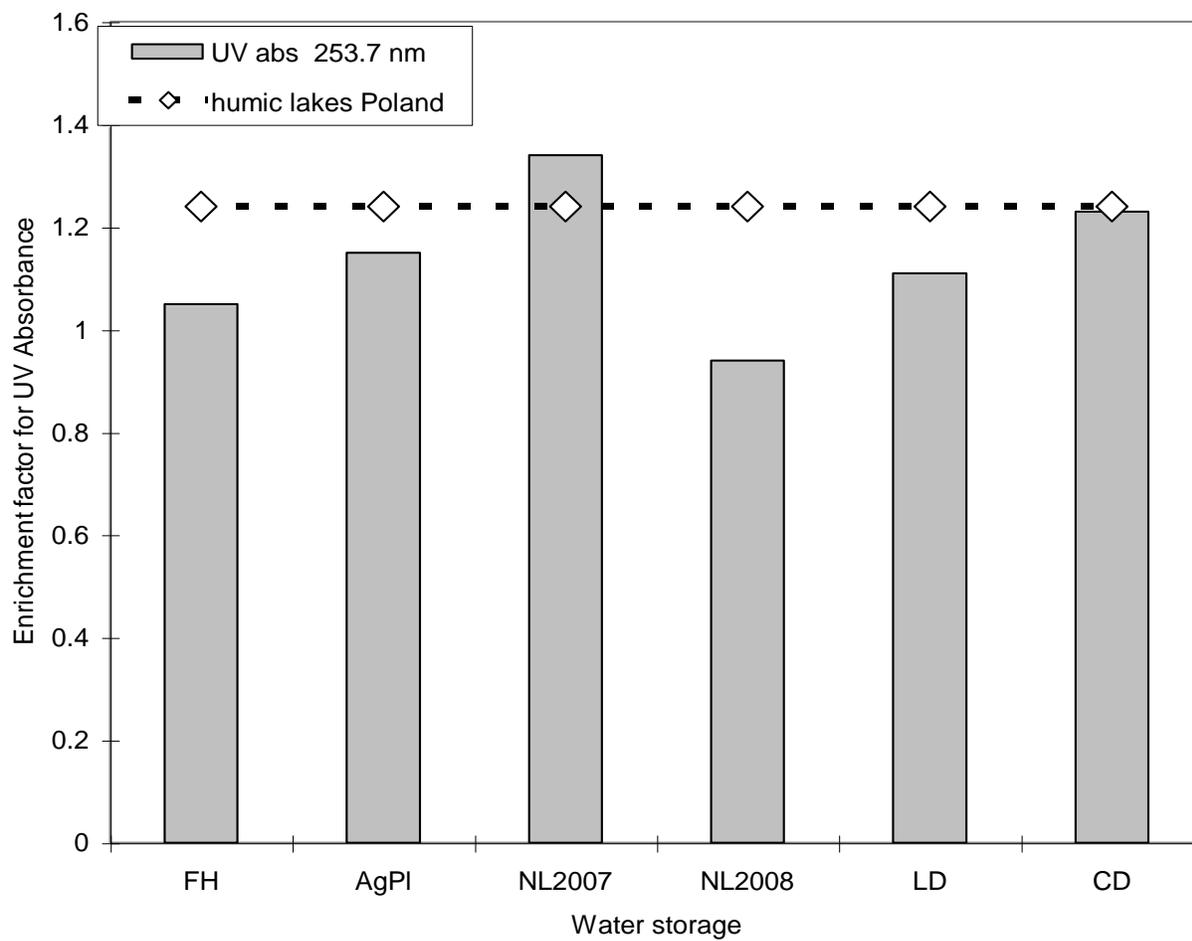
Figure 2: A comparison of the enrichment factors for UV absorbance at 253.7 nm for five of the six southeast Queensland water storages, and the highest value recorded for a humic lake in Poland (data from Kostrzewska-Szlakowska 2005). FH is Forest Hill, AgPl is the Agricultural Plot tank at the University of Southern Qld, NL is Narda Lagoon sampled in 2007 and in 2008, LD is Lake Dyer, and CD is Cooby Dam. There are no results for Alderton due to interference from suspended clay particles that passed through the glass fibre filter paper.

Figure 3: A comparison of the permanganate oxygen demand recorded for microlayer (mic) and subsurface (subs) water samples from six water storages in southeast Queensland, and the highest subsurface value recorded for six humic lakes sampled in Norway in mid-summer (data from Hessen 1985). FH is Forest Hill, AgPl is the Agricultural Plot tank at the University of Southern Qld, NL is Narda Lagoon sampled in 2007 and in 2008, LD is Lake Dyer, CD is Cooby Dam Ald is the Alderton water storage.

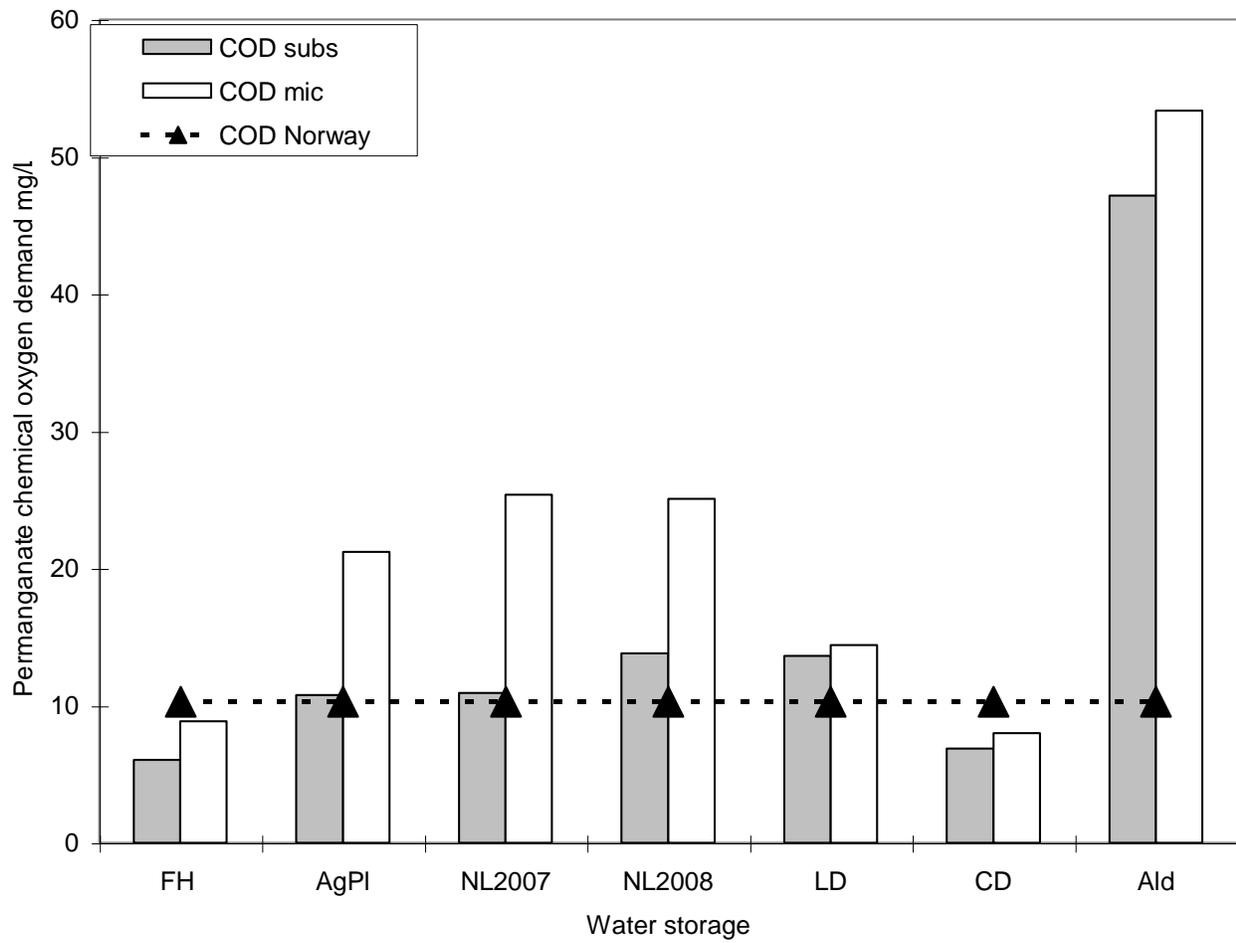
Figure 4: A comparison of the population density of phenol-degrading bacteria and the ultraviolet light absorbance (abs 253.7 nm) of microlayer (micro) and subsurface (subs) water samples from five water storages in the Lockyer Valley, southeast Queensland, sampled in February and March 2009. Bacterial populations were quantified using the most probable number method (MPN), the indicator dye resazurin and phenol as the sole carbon source. Results were expressed as colony forming units (CFU) per 100 mL of sample. NL is Narda Lagoon, LD is Lake Dyer, Logan is Logan's storage, CU and CL are Caffey upper and Caffey lower dams respectively.



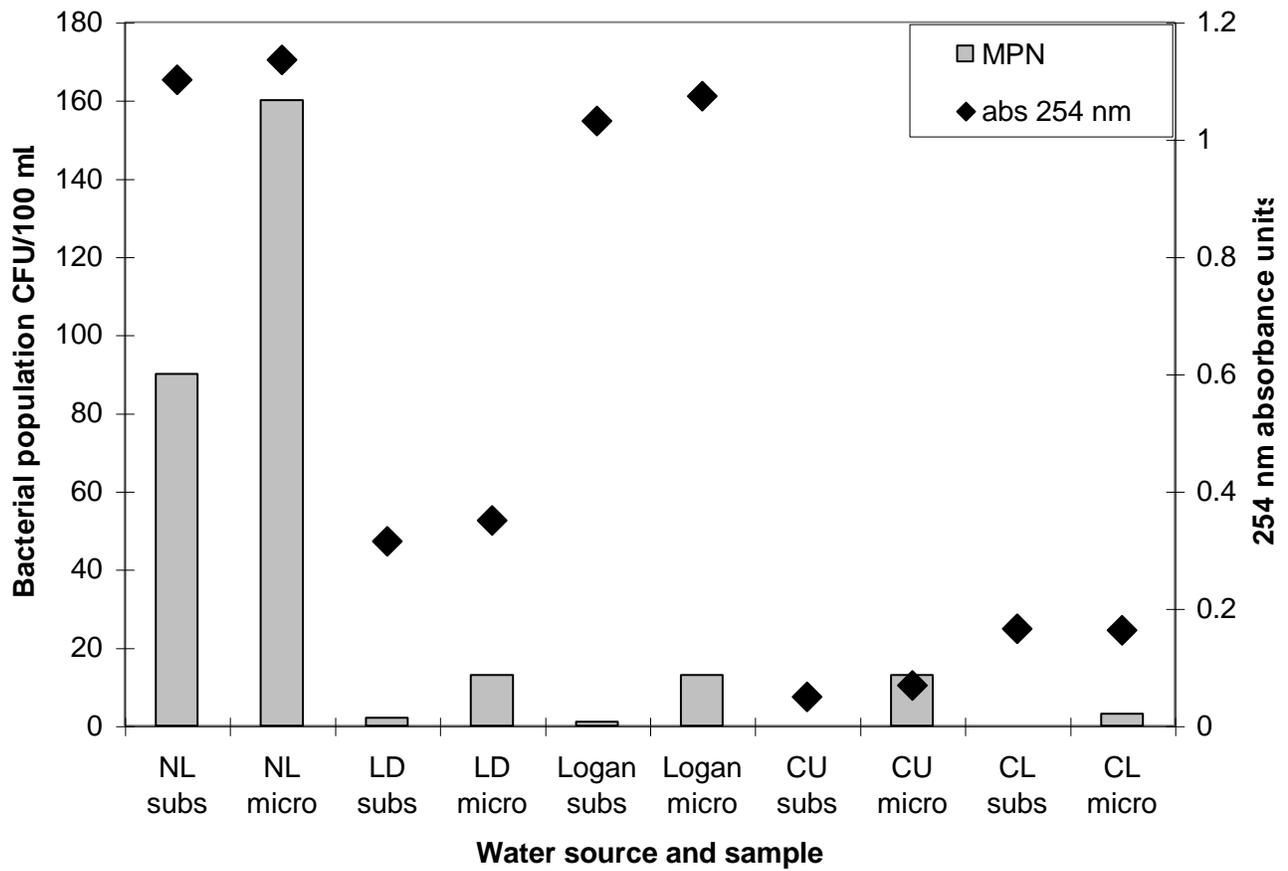
**Figure 1**



**Figure 2**



**Figure 3**



**Figure 4**