The use of algal-bacterial biofilms to enhance nitrification rates in lagoons: experience under laboratory and pilot-scale conditions

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Abstract
Investigations were undertaken at the Western Treatment Plant (WTP), near Melbourne, Australia, to find ways of increasing overall ammonia and nitrogen removal rates in the WTP lagoon systems. Immobilisation of nitrifying bacteria in biofilms was one approach explored. Preliminary tests showed that algal/bacterial biofilms capable of achieving ammonia removal rates of 3 to 4 µg N/cm² · h would form on support surfaces immersed in the WTP lagoons. A laboratory-scale investigation was carried out to characterise the influence of parameters such as pH, temperature, COD level, dissolved oxygen concentration and incubation depth on biofilm performance. This study was followed by a pilot-scale investigation in a series of experimental ponds at the WTP. This compared the performance of three ponds, two containing 9360 m² and 18240 m² respectively of a geotextile biofilm support material and one containing no biofilm support material (the control pond). Ammonia removal rates comparable to those obtained in the preliminary tests were obtained when the biofilm support material was within the top 500 mm of the lagoon, i.e. in the photic zone. COD and suspended solids levels in the effluents from the biofilm containing ponds were substantially lower than those in the control pond effluent.

Keywords: Algae; ammonia; biofilm; lagoons; nitrification; nitrogen

Introduction
Much of the wastewater generated in Melbourne, Australia is treated in two major treatment plants. The larger of these two plants is the Western Treatment Plant (WTP), formerly known as Werribee Farm or the Werribee Treatment Complex. This plant occupies an area of 10,150 ha on the western shore of Port Phillip Bay, the bay around which the city of Melbourne has grown up. It currently handles around 500 ML/day dry weather flow of a mixed domestic/industrial wastewater. Seventy percent of the flow entering the plant is treated in a series of multi-pond lagoons covering an area of 1650 ha; the balance goes to land filtration or grass filtration. The effluent from these treatment processes is discharged into Port Phillip Bay through four discharge points. These are subject to licence conditions imposed by the Environment Protection Authority of Victoria.

Sizeable amounts of nitrogen (4000 tonnes/year at present), much of it in the form of ammonia, enter Port Phillip Bay from the WTP. Since the 1970s there have been concerns about the impact this nitrogen might be having on Port Phillip Bay’s ecosystems. These concerns prompted a continuing series of investigations into ammonia and nitrogen removal processes at the WTP. These investigations had two goals: firstly, to improve our understanding of these processes and, secondly, to identify ways of reducing the ammonia and total nitrogen content of the WTP discharges.

Prospects for increasing nitrogen removal rates in the land and grass filtration processes were limited, so attention was focussed on the lagoon systems. Early studies indicated that nitrification played an important role in reducing the ammonia content of the lagoon waters. If nitrification were followed by denitrification, a sizeable reduction in the total
nitrogen content of the lagoon waters was also observed. The key step in this coupled nitrification/denitrification process was felt to be nitrification. Even in the absence of denitrification, nitrification still helped reduce ammonia levels. In addition, nitrified waters were observed to undergo a degree of denitrification even in the more aerobic ponds. This was inferred to take place in anoxic parts of the sediments, where high concentrations of denitrifying organisms are present (Morrison, 1984).

For much of the time, nitrification rates in the lagoons remain at a comparatively low level, with active nitrification apparently confined to the upper sediments of aerobic ponds. On occasions, however, large nitrifier populations become established in the water column of a lagoon and persist there for a while. Under such conditions, much higher nitrification rates and ammonia removal rates are observed. However, these periods of intense nitrification are usually short-lived, unpredictable and confined to the hotter months.

Prolonging these periods of high water column nitrifier activity looked an obvious way to increase overall ammonia and nitrogen removal rates in the lagoons. However, before such an approach could be made to work, a way of establishing and maintaining large water column nitrifier populations had to be found. One approach involved placing biofilm support surfaces in the lagoons to create an immobilised nitrifier population. The feasibility of doing this was tested in a pilot-scale study (Constable et al., 1989). Pieces of car tyre, PVC sheeting and polypropylene fishing net were the materials used in this study. When incubated in one of the lagoons, these materials all developed surface biofilms comprising a mixture of algae and bacteria. The appearance of biofilms from different depths varied considerably. Nevertheless, all of them achieved surface nitrification rates of at least 3 to 4 $\mu$g N/cm$^2 \cdot$ h.

These observations confirmed that it was possible to establish immobilised nitrifier populations in ponds. However, the large scale practicability of this approach needed confirming. To do this, it was first necessary to gain an understanding of how factors such as temperature, dissolved oxygen (DO) level and pH affected the performance of these nitrifying biofilms. At the WTP, these factors fluctuate in an uncontrollable way, making it practically impossible to obtain the required design information from in situ studies. For this reason the laboratory study described below was initiated.

**Laboratory biofilm study**

The experimental facility used in this study comprised eight PVC tanks, each of a nominal capacity of 62 litres. Each tank was 550 mm long and 450 mm wide, and was filled with water to a depth of 250 mm. Distributed across the floor of each tank were several air diffusers of the type used in aquariums; the air introduced through these diffusers helped supply microbial oxygen needs as well as keeping the tank contents well mixed. Fluorescent lamps were used to make light conditions in the laboratory more like those in the field. Two tanks were set up in a constant temperature room so that the influence of temperature on biofilm performance could be studied; the others were maintained at 20±1ºC, with the help of a waterbath.

The biofilm supports used in this study were PVC plates, each 400 mm wide, 200 mm deep and 1.5 mm thick. These plates were suspended vertically in the tanks with their top edges 20 mm below the water surface and their bottom edges 30 mm above the tank floor. On occasions, single plates were replaced by composite plates containing three individually removable horizontal strips; these were used to study the influence of depth on biofilm performance. Five tanks were equipped with plates; these tanks, designated T2, T5, T6, T7 and T8, were fitted with 14, 6, 12, 24 and 17 plates respectively. The remaining tanks were used as controls, with tanks T1 and T3 being run purely as suspended growth reactors; these tanks contained no support plates.
To ensure the microbial populations in the tanks had a similar mix of species to those in the WTP lagoons, the tanks were inoculated with water collected from these lagoons. In addition, biofilm-coated plates from Constable’s original study site were placed in each tank. These were removed once the microbial populations in the tanks appeared well established.

The series of eight tanks was operated continuously for over two years. Over this period the influence of the following parameters on biofilm performance was studied: temperature, pH, hydraulic residence time, influent composition (both COD and ammonia levels were varied), extent of illumination, dissolved oxygen. More extensive information on the experimental program, operating procedures, analytical techniques and the results of the study can be found in Baskaran (1995) and Baskaran et al. (1992).

The laboratory study confirmed Constable’s (1998) findings that actively nitrifying algal-bacterial biofilms could be developed on surfaces within lagoons. It also showed that these biofilms were robust and able to perform effectively over a range of temperatures, pH levels, DO levels, and influent COD and ammonia loadings. In addition, it was evident that, under conditions conducive to nitrification, ammonia removal rates as high as 8 µg N/cm²·h could be achieved. These findings were encouraging, and a pilot-scale study of this biofilm-based approach to enhancing nitrification was initiated. Details of this study are given below.

Pilot-scale study

Close to lagoon 115E, a large, comparatively new lagoon at the WTP, is a set of four experimental ponds. Each pond is roughly one twentieth the size of a normal WTP pond. These experimental ponds are each 260 metres long, 23 metres wide and when full have a surface area of 5880 m². The depths of the ponds vary, being 0.6 m in pond 1, 1.2 m in ponds 2 and 4, and 2.3 m in pond 3. Ponds 2 to 4 were used in the pilot-scale study, with biofilm support materials being placed in ponds 2 and 3 and pond 4 being run as a control.

Selection of a suitable biofilm support material was a far from trivial exercise. To obtain meaningful information on the performance of biofilm augmented ponds, the drop in ammonia concentration across the ponds needed to be around 10 mg/l. It was planned to operate the ponds at a hydraulic retention time of 7 days, which is fairly typical for the ponds at the WTP under normal conditions. The design was based on an ammonia removal rate of 3 to 4 µg N/cm²·h, as obtained by Constable et al. (1989) and also by Baskaran under low DO (2–3 mg/l) conditions (Baskaran et al., 1992). On this basis, around 10,000 m³ of support surface would be required in pond 2 and double that amount in pond 3. This meant that the material used would have to be cheap, readily installed and not too bulky. It would also have to be readily colonised by biofilm forming organisms and capable of retaining its biofilm under all weather conditions.

Initially, trials were undertaken with a variety of inexpensive plastic materials. Samples of these were placed for 3 months in pond 3 of lagoon 115E and their condition monitored regularly. A combination of factors influencing their suitability as biofilm supports was assessed; these included: the rate of biofilm development; the luxuriance or sparseness of the final biofilm; its nitrifying capability; its strength of attachment to the support material; the long term tensile strength of the material under conditions prevailing in the lagoons; and how easily the material could be installed and maintained in a vertical position in the lagoon water column.

As might have been expected, materials with a textured surface proved superior to smooth surfaced materials. The nitrification rates achieved by the biofilms on the various textured materials were similar so the final selection of a support material was based on other criteria. The material chosen was a polypropylene geotextile (Polyfelt TS1600) that...
had neutral buoyancy and a higher tensile strength than the other materials. More details on the materials and test procedures can be found in McLean (1999).

The geotextile material was made up into panels that were attached to 240 m long support wires spaced equally across the ponds. In pond 2 the panels were 500 mm wide and 500 mm deep; they were arranged in 38 rows, spaced 600 mm apart, to give a nominal surface area of 9360 m². In pond 3 the panels were 800 mm wide and 2 m deep; in this case there were 19 rows, spaced 1200 mm apart and the nominal surface area was 18,240 m². This longitudinal arrangement was chosen so as to minimise hindrances to wind induced mixing, an important feature of the full-scale ponds at the WTP and one that it was desirable to reproduce in the experimental ponds. In the full-scale ponds, the wind induces a surface flow in the wind direction and a sub-surface return flow. The mixing this causes is important in preventing short-circuiting and stratification (Chapman, 1988).

This need to maintain effective mixing was also the reason why the support wires were run at a depth roughly equal to one third of the distance between the water surface and the pond base. At this depth one is in the zone where the opposing surface and subsurface flows intergrade (Chapman, 1988); water velocities here are low and it was hoped that this would keep any disruptions to wind induced mixing to a minimum.

The different sizes of the panels in the two ponds meant that different installation methods had to be employed. In pond 2, each of the 500 mm high panels had an envelope of fibre reinforced PVC material stitched to its upper edge. A 20 mm by 20 mm strip of closed cell polyethylene foam was inserted into this envelope. The buoyancy provided by this foam was sufficient to keep the panel vertical in the pond. In pond 3, the same means was used to buoy the upper edge of the panels. However, in this case the panels were larger and the panel support wire was attached close to the middle of the panel rather than at its lower edge. To keep the lower portions of these panels vertical, a seam was created along the lower edge of the panel and a weight (a length of wire) inserted.

Operation of the experimental ponds commenced in September 1994 and regular monitoring was carried out until late 1997. Influent to these ponds was drawn either from pond 3 or pond 5 of lagoon 115E (a 10-pond lagoon system), depending on the season and the COD levels in these two ponds. Pond 3 was the usual source in summer and pond 5 in winter. Problems affecting lagoon 115E between November 1996 and May 1997 meant that influents to the ponds were atypical over this period; data obtained during this time were therefore disregarded when the results of the study were analysed.

Flows to individual ponds were measured using calibrated square notch weirs and controlled to give residence times of 7 days (initially) and 14 days (later). The influent flowrate, influent and effluent pH, influent and effluent DO and influent and effluent temperature were all measured continuously and recorded on a Unidata Macrologger data acquisition system. Every four days, samples were taken from the influent and from the effluent of ponds 2 to 4. These samples were analysed and values of the following parameters determined: BOD, COD, alkalinity, chlorophyll, suspended solids, ammonia, nitrite, nitrate and TKN. Details of the analytical procedures and other relevant information can be found in McLean (1999).

Results and discussion
The ammonia-N content of the influents to the experimental ponds was usually around 40 mg/l. Organic nitrogen levels of around 8 or 9 mg/l were typical while nitrite and nitrate levels were low. This was to be expected as conditions in the early ponds of the WTP lagoons are not conducive to nitrification. The influent COD, averaged over successive three-monthly periods, varied from as high as 168 mg/l down to 104 mg/l, with most values being nearer the lower end of this range (McLean, 1999). Temperatures in the ponds...
followed typical seasonal trends at the WTP, with a low of around 9°C in winter and a high of 25°C in summer.

The dissolved oxygen levels in the ponds followed a diurnal cycle, with a low point around 6 to 8 a.m. and a peak at around 4 p.m. Depending on the season and other factors, lows ranged from 1 to 3.5 mg/l while the highs ranged from 2 to 5.5 mg/l. Based on the work of Baskaran (1995), these are adequate for nitrification provided other factors influencing nitrification rates are favourable. The pH certainly appeared favourable, with an average daily value of about 7.7 and a range of from 7 to around 8.7; these are well above the levels at which pH appeared to have any significant impact on nitrification (Baskaran, 1995). The relatively favourable pH levels were not altogether unexpected as past experience at the WTP has indicated that its wastewaters are very well buffered.

The effectiveness of the experimental ponds at removing ammonia is well illustrated in Figure 1. This shows quarterly average ammonia concentrations in the influent to the ponds and in the effluents from each of the three experimental ponds (designated EP2, EP3 and EP4). Little was achieved in the first three months when the biofilm was presumably becoming established. This was also the coldest time of the year and the one when nitrification rates at the WTP are normally at their lowest. Once the biofilm was established, the best performing pond (EP2) quite frequently achieved reductions in ammonia-N of around 10 mg/l or greater, mostly during the hotter months. The average reduction in EP2 over the relatively normal two-year period of operation from October 1994 to September 1996 was 8.7 mg/l. This was an encouraging result, given that the reduction predicted using the original design nitrification rate is 9.3 mg/l.

The performance of pond EP3 was much less encouraging. Over the same two-year period, the average reduction in ammonia-N in this pond was only 4.8 mg/l, well below the figure of 8.7 mg/l obtained in pond EP2. The most likely explanation for this difference is the difference in biofilm support arrangements between the two ponds. In pond EP2 the geotextile panels extend to a depth of only 500 mm, whereas in EP3 they reach down a full two metres below the surface. Only the top 500 mm or so of the WTP lagoons is penetrated by light (McLean, 1999). Therefore, whilst the panels in EP2 receive incident light over their entire area, three-quarters of the panel surface in EP3 lies in a region receiving little or no light. Light is essential for the growth of algae, which make possible the high nitrification rates attainable in algal/bacterial biofilms (Baskaran, 1995). Therefore much of the panel surface in EP3 would have been deficient in algae and unable to nitrify well.

Figure 1 Average quarterly ammonia concentrations in the influent and experimental pond effluents
This explanation is supported by the observations of both Constable (1988) and Baskaran (1995). Both found that biofilm nitrification rates decreased with increasing depth while at the same time the biofilm became browner and thinner. Further support comes from McLean (1999), who showed that surface nitrification rates for EP3, when calculated on the basis of the panel area in the illuminated (photic) zone alone, are comparable with those for EP2. The practical implications of this finding are that biofilm support surfaces need to be placed in the upper 500 mm of the lagoon water column if they are to be fully effective.

Also of interest in this study was the performance of the control pond, EP4. As Figure 1 shows, this achieved an ammonia removal performance that at times was comparable with that in pond EP2. For the two-year period October 1994 to September 1996, this pond achieved a mean ammonia-N reduction of 5.9 mg/l, significantly less than that achieved by pond EP2 but better than that in EP3. This demonstrates what can be achieved if a high suspended growth nitrifier population is maintained in the WTP lagoons.

The difficulty of maintaining such a population in the full-scale lagoons at the WTP has generally been blamed on the surges in lagoon inflows that follow heavy rains. Because nitrifiers reproduce comparatively slowly, washout of water column nitrifier populations could easily occur during such surges. Indirect support for this explanation comes from observations of the behaviour of the control pond EP4. This pond was well protected against hydraulic surges and its nitrifier populations, unlike those in typical WTP lagoons, maintained a high level of activity throughout the study period.

One obvious area of difference between pond EP4 and the other two ponds was in the colour and clarity of their effluents. Pond EP4 looked greener and appeared to have a larger suspended algal population. These observations are borne out by the quarterly average TSS concentrations shown in Figure 2. Based on these values, over the two-year period October 1994 to September 1996 pond EP4 achieved an average effluent TSS concentration of 21.5 mg/l whereas ponds EP2 and EP3 got down to levels of 16.3 and 16.0 mg/l respectively. This corresponds to a drop in TSS concentration across pond EP4 of 6.8 mg/l, compared to drops across EP2 and EP3 of 12.0 and 12.3 mg/l, respectively.

Quarterly average chlorophyll concentrations in the three ponds confirm that the differences in TSS levels are largely due to algae. For the two-year period referred to above, mean total chlorophyll concentrations were: in the pond influents, 172 µg/l; in ponds EP2 and EP3, 145 and 142 µg/l; and in pond EP4, 247 µg/l. If the chlorophyll content of dry algal biomass is taken to be 1.5% (a figure obtained from other work at the WTP), this...
difference of 100 µg/l translates into a TSS difference of around 6 to 7 mg/l. This result is very close to the difference in average effluent TSS concentrations between pond EP4 and the two biofilm ponds. The increased algal content of pond EP4 also helps explain the higher COD levels in that pond’s effluent (a mean of 112 mg/l) compared to those for ponds EP2 and EP3 (means of 99 and 102 mg/l respectively).

The low levels of TSS in the two biofilm ponds are consistent with observations made in Baskaran’s (1995) laboratory study. Baskaran planned his experimental study in the expectation that suspended microbial populations would contribute substantially to the performance of the tanks containing supported biofilm. His system of three control tanks was designed to operate in such a way that the contribution of the immobilised microorganisms in his tanks could be separated from that of the suspended fraction. However, it rapidly became apparent, both from visual inspection of the tanks and from the appearance of the tank effluents, that there was very little suspended growth present in tanks when biofilm support plates were present. This contrasted strongly with conditions in the tanks that lacked biofilm supports.

Why tanks containing a sizeable immobilised algal/bacterial population should have such a low suspended microbial population has yet to be fully resolved. However, its practical implications are considerable. For ponds lacking supported biofilms, a strong correlation has been shown between the occurrence of high algal concentrations and the development of high nitrifier levels in the water column. Observations in tank EP4 (McLean, 1999), previous studies of lagoon management techniques in the experimental ponds (Gross et al., 1994), and an analysis of monitoring data for lagoon 115E (Hurse and Connor, 1997) have all confirmed this link. It would appear that, in ponds lacking an immobilised nitrifier population, high nitrification rates are only achievable when algal concentrations, and hence effluent TSS levels, are high. In ponds containing an immobilised nitrifier population, however, this problem is largely obviated.

In the discussions above, a number of comparisons have been drawn between the pilot-plant studies of McLean (1999) and the laboratory-scale experiments of Baskaran (1995). When comparing results obtained in systems as dissimilar in size as these were, the validity of such comparisons is always a concern. One potentially significant difference between the systems lies in the amount of biofilm surface area present per unit tank volume. For the pilot-scale studies the figure was 1.3 m²/m³ whereas for the laboratory tanks it ranged from 15.5 to 62 m²/m³. This does not represent a disadvantage for the pilot-scale ponds as tanks with high area to volume ratios would be more susceptible to mass transfer limitations. These limitations were largely overcome in the laboratory experiments by the use of air diffusers, so differences between the pilot and laboratory systems were probably smaller in practice than the above figures would suggest.

It is also instructive to compare various parameter loadings on these two systems. What basis to use in determining loadings is often open to question and somewhat arbitrary, so the ones used in the following sections are those that seemed most appropriate to the authors.

Ammonia loadings can influence nitrification rates, which could be expected to fall away at low ammonia concentrations. In McLean’s (1999) study, pond influents contained around 40 mg/l of ammonia-N, which translates into a loading of 4.5 mg NH₄−N/m²·day for the ponds containing biofilm. This is well above the loadings on Baskaran’s (1995) tanks, which ranged from 0.36 to 1.94 mg NH₄−N/m²·day, depending on the hydraulic retention time (either 3 or 4 days) and the number of biofilm support plates. It can be inferred from these figures that availability of ammonia should not have been a significant constraint in the pilot-scale work.

COD loadings are also important since, at the WTP at least, there appears to be a threshold BOD level above which nitrification is strongly inhibited (Constable, 1988). In
lagoons, COD loadings are often expressed in units of kg COD/ha (of pond surface) · day. On this basis, the loadings on ponds EP2 and EP3 (190–290 and 380–580 kg COD/ha·day) are much higher than those in Baskaran’s (1995) tanks (56–117 kg COD/ha·day). In this case, however, a more suitable basis for comparison would seem to be kg COD/m³ (of pond volume) · day. Using this basis brings the systems much closer together: for the pilot-scale ponds, loadings range from 0.016 to 0.024 kg COD/m³ · day, while for the laboratory tanks the range was 0.023 to 0.047 kg COD/m³ · day.

Conclusions
The studies described above confirm the technical feasibility of establishing immobilised nitrifier populations in the WTP lagoons. They showed, however, that nitrification rates of the order of 3 to 4 µg N/cm² · h were achievable only if the biofilm support surface was confined to the top 500 mm of the lagoon. Surface extending below this zone receives almost no light and the biofilm that develops is deficient in algae and markedly less effective at nitrification.

The control pond achieved an ammonia removal rate around two-thirds of that obtained in the better performing biofilm pond. It maintained a comparatively high nitrification rate throughout the experimental period and demonstrates what can be achieved under conditions where the water column nitrifier population is protected from the effects of hydraulic surges and other adverse influences.

The results confirmed the overall superiority of the better performing biofilm pond over the suspended growth control pond. The ammonia removal rate was 50% better and the effluent TSS level was also substantially lower.

Acknowledgements
The financial support provided to the authors by the Urban Water Research Association of Australia is gratefully acknowledged. The authors are also most appreciative of the financial and other support received from Melbourne Water and its predecessor, the Melbourne and Metropolitan Board of Works.

References


