Laboratory modelling of manganese biofiltration using biofilms of *Leptothrix discophora*

Running title: Leptothrix

C. K. Hope[1*] and T. R. Bott[1]

[1]School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham. B15 2TT. United Kingdom.

[*]Correspondence to present affiliation: Department of Microbiology, Eastman Dental Institute for Oral Healthcare Sciences, University College London, 256 Gray's Inn Road, London. WC1X 8LD. Tel: +44 (0)207 915 1050. Fax: +44 (0)207 915 1127. email: chope@eastman.ucl.ac.uk

Updated on 3/12/2007 to Dr Chris Hope, Lecturer in Oral Biology, School of Dental Sciences, University of Liverpool, Edwards Building, Daulby Street, Liverpool. L69 3GN. Tel: +44 (0)151 706 5252 Fax: +44 (0)151 706 5809 e-mail: c.hope@liv.ac.uk

Abstract

Laboratory biofilters (pilot-scale, 20 litres and laboratory-scale, 5 litres) were constructed in order to model the bioaccumulation of manganese (Mn) under flow conditions similar to those occurring in biofilters at groundwater treatment sites. The biofilters were operated as monocultures of *Leptothrix discophora*, the predominant organism in mature Mn oxidising biofilms. Biologically mediated Mn bioaccumulation was successfully modelled in both filter systems. The data obtained showed that in the small-scale biofilter, the Mn concentrations that gave the highest rate of Mn bioaccumulation, shortest maturation time, highest optical density (biomass) and growth rate were between

2 000 and 3 000µg l^{-1}. The non-problematic scale-up of the process from the laboratory-scale to the pilot-scale biofilter model suggests that Mn biofilters may be 'seeded' with laboratory grown cultures of *Leptothrix discophora*. By initially operating the biofilter as a re-circulating batch culture, with an initial Mn concentration of ~2 500µg l^{-1}, it is hoped to reduce the filter maturation time from months to days.

Key Words

Leptothrix discophora, biofilter, groundwater, manganese, bioaccumulation, biofilm

Abbreviations Used

Mn Manganese

[Mn] Manganese concentration

1. Introduction

Manganese (Mn) is frequently found in potable groundwater in the soluble, divalent (Mn^{2+}) or quadrivalent (Mn^{4+}) form. Although ingestion of manganese, through drinking water, at concentrations of up to 500µg l^{-1} has no harmful effects upon human health (WHO 1993), its presence in drinking waters at concentrations above 100µg l^{-1} is undesirable to customers due to discolouration of the water and the subsequent staining of laundry and plumbing fixtures. The European Environmental Committee (80/778/EEC) recommends an upper limit of 50µg l^{-1} for Mn in water for drinking.

A filter able to remove Mn from groundwater by biological oxidation and accumulation (bioaccumulation) without the addition of chemicals is said to be a mature

filter (Vandenabeele 1992). A mature biological filter capable of Mn removal is achieved by passing aerated water vertically through a column of filter sand, in the absence of chlorine, and allowing the benthic microorganisms to proliferate as biofilms within the void spaces (Bourgine at al 1994). Biological treatment, for the bioaccumulation of Mn in slow sand filters has been reported to lower Mn concentrations successfully from an input of 350µg l^{-1} to an output of 20µg l^{-1} (Seppänen 1992). Maturation times for these filters can vary from weeks to months, even after seeding with filter medium from other mature filters (Mouchet 1992). At the Saints Hill water-treatment plant (South East Water Ltd, Kent, UK.), having raw groundwater Mn concentrations between 500 and 600µg l^{-1}, manganese biofilters were operated for three months before Mn was removed by biological processes (Bourgine et al 1994). The success of manganese biofilter maturation can differ greatly between two water treatment sites, even though the chemical and biological characteristics of the incumbent groundwater may be very similar (Ward 1994a).

There is an urgent need to improve the ability to establish mature Mn biofilters in a reasonable time, at suitable water treatment sites. In order to overcome some of the difficulties associated with this requirement a pilot biofilter was designed and operated using a monoculture of *Leptothrix discophora*, thereby allowing a direct comparison of the effects of changing process variables to be made. The principal objective of the work was to establish the conditions which would reduce filter maturation times to a minimum.

Leptothrix discophora is one of the predominant organisms in Mn biofilters (Czekalla et al 1985, Vandenabeele et at 1995) and is characterised by its ability to oxidise Mn^{2+} (Ghiorse 1984) and to form a sheath incorporating manganic oxides

(Mulder 1981). It is this ability to oxidise Mn as well as iron that distinguishes *Leptothrix* species from other sheath forming genera, such as *Sphaerotilus*, which can only oxidise iron (Fe) (Corstjens et al 1992).

Leptothrix discophora strain SS-1 (ATCC 43182) is a Mn oxidising, aerobic, heterotrophic gram negative short rod, which has lost is sheath forming ability after successive inoculations onto laboratory media at high growth rates. Its inability to form a sheath is possibly due to the loss of a plasmid encoding sheath genes (Hope 1999). This strain has received the most attention in terms of its growth kinetics, metabolism and Fe/Mn oxidation (Adams and Ghiorse 1986, 1987, Boogerd and DeVrind 1987, DeVrind-DeJong at al 1990, Zhang 2002).

'Wild-type' *Leptothrix discophora* are most probably the sheath forming strain SP-6 (ATCC 51168). Wild-type *Leptothrix discophora* appear as two indistinct forms; motile (sheathless) cells and immotile (sheathed) cells. Sheath-forming strains of *Leptothrix discophora* (SP-6) can be maintained indefinitely in slow growing conditions at temperatures between 20 and 25ºC in combination with careful culture management at 4ºC and -80ºC (Emerson 1992).

Biofiltration of groundwater was modelled in batch cultures of *Leptothrix discophora* SP-6 grown in laboratory fermenters containing a side arm loop passing medium, supplemented with Mn, through a biofilter. The decrease in the amount of detectable Mn over time in these systems represented the bioaccumulation of manganese by the biofilms of *Leptothrix discophora* within the filter matrix. Modelling biological filter processes at the laboratory scale allowed the effects of defined parameter changes to be transferred to full-scale biofilters (Rowan 2002). By successfully modelling

biofiltration and studying Mn bioaccumulation rates (i.e. the decrease in Mn concentration in water passing through the filter) in these filters we hoped to elucidate optimum operating parameters for the process and reduce filter maturation times from months to days.

2. Materials and methods

2.1. Small-scale biofiltration model

A small-scale biofiltration model was designed consisting of a 5 litre Quickfit VisiFlow® (QVF) fermenter (QVF process systems Ltd., Stafford, UK.) operating as a mixing vessel with an external loop circuit (Figure 1) forming the biofilter. The external loop comprised of a specially constructed section of glass tubing, 26mm in diameter. This was filled to a depth of 0.3m with washed, grade 8/16 sand (~0.4mm diameter grains), a standard building material. The maximum flow rate available from the peristaltic pump (Watson-Marlow Bredel Pumps Ltd., Falmouth, UK.) was 48ml min^{-1}, equating to a cross sectional flow velocity of 0.09m min^{-1} through the filter. The residence time of the system at maximum flow was 1.74h.

Air was supplied to the system via a hydrator to help reduce the effects of evaporation from the mixing vessel by pre-saturating the incoming air. From experience, this method of water retention was more effective than conventional condenser technique. A sparger was included in the mixing vessel air supply to reduce bubble size, to maximise mass transfer area and to assist mixing. Sparging allowed the optimisation of air flow (2 l min^{-1}) to oxygen transfer rates based on experience. Higher rates of air flow formed ‘air slugs’ due to the coalescence of smaller bubbles which would reduce the rate

of oxygen transfer. During the commissioning of the both biofilters, dissolved oxygen probes were incorporated into the mixing vessel. Upon initiation of air flow into the mixing vessels (see materials and methods), 100% oxygen saturation (8.2 mg l^{-1} at 26ºC) was soon achieved. This was maintained indefinitely after inoculation with *Leptothrix discophora* (data not shown). A magnetic stirrer, rather than an impellor was employed in the mixing vessel in order to maintain the biological integrity of the equipment. The resulting mixing was considered to be effective based upon a visual test assay after adding Coomasie blue to the system.

The mixing vessel was filled with 5 l of buffered peptone yeast glucose (PYG) medium (Adams 1985); 0.25g l^{-1} Peptone from enzymatic digest of soya (Fluka, Sigma-Aldrich, Buchs, Switzerland), 0.25g l^{-1} Yeast extract (Fluka), 0.25g l^{-1} Glucose (Fisher Scientific UK, Loughborough, UK), 0.6g l^{-1} $MgSO_4$ (Fisher), 0.07g l^{-1} $CaCl_2$ (Fisher), 3.57g l^{-1} N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Fluka). The pH of the medium was raised to 7.6 with 1M KOH (Fisher) which fell to between 7.0 and 7.2 after autoclaving. PYG medium was made up in sterile reverse osmosis (SRO) water, with v/v 1% chlorine free tap water added to provide trace elements. $MnSO_4$ was added separately as a solution in 20ml of SRO water which was acidified with 1ml of 5M H_2SO_4 (Fisher) after autoclaving. The total mass of $MnSO_4$ added to the system varied as an experimental parameter. Before operation, the entire small-scale biofilter apparatus containing PYG medium, was autoclaved at 121ºC for 30 minutes.

A conical flask containing 100ml of buffered PYG medium was inoculated with 20ml of *Leptothrix discophora* SP-6 suspension, from frozen stocks stored at -80ºC. It should be noted that the frozen stocks did not include glycerol as a cryo-protectant since

this glucose homologue may cause cell lysis in *Leptothrix discophora* at concentrations above 0.05 % w/v (Adams 1986). The inoculated flasks were grown as shake cultures at 25ºC. 20ml of these cultures were added to the mixing vessel after 5 days of growth, following the development of turbidity (i.e. during logarithmic growth phase).

After inoculation of the biofilter with *Leptothrix discophora*, a brief period of mixing was allowed (1 minute) and a 23ml sample was drawn from the rig by hypodermic syringe via a rubber septum. Further samples were taken from the system, typically every hour. These samples were transferred to sterile plastic containers and acidified by the addition of 1ml of 5M H_2SO_4. Acidification prevented air induced oxidation of the Mn and adsorption onto the walls of the plastic container. Additional PYG medium containing Mn, at a concentration suitable to acheive the desired Mn concentration in the system, were introduced into the mixing vessel, when required, to maintain available nutrients and the volume of liquid in the mixing vessel above the level of the external loop inlet.

During and experiment, the temperature was monitored via a thermometer inserted into a holder located in the liquid. The rig was operated at a steady 26ºC, provided by the heating effects from the magnetic stirrer (New Brunswick Scientific, Edison, NJ, USA.). Temperature control was available by a tap water cooling finger, the flow into which was controlled manually when necessary.

2.2. Large-scale biofiltration model

The majority of the large-scale biofiltration model was contructed from 1-inch, QVF glassware components (Figure 2) with a 10 litre QVF fermenter serving as a mixing

vessel. The biofilter itself was custom made from a section of 75mm diameter QVF tubing, 1.5m long with a series of six sample points placed along the length of the filter. The available length of the biofilter containing filter medium was 1m. The filter packing medium was supported by a 9mm thick glass plate containing nine, 6mm holes arranged in a regular pattern (see Figure 2, filter medium support plate). The filter medium used in these experiments was 25mm (described as 1-inch) pebbles, a standard building material. Flow was provided by a Lowara Little Giant centrifugal pump (Lowara UK Ltd, Axminster, UK.) which was capable of generating a maximum sustainable flow rate through the system estimated as 20 l min^{-1}. A loop of the QVF connecting sections was made the highest point in the system to prevent the liquid draining through the biofilter due to gravity. The residence time of the system was typically 2.5mins.

The entire system was sterilised before use by connecting to the laboratory steam supply for at least twenty four hours. The thermometer in the biofilter typically recorded a temperature of ~102ºC throughout the steaming process. A pH probe (Ingold Inc, Urdorf, Switzerland) was autoclaved separately and placed *in situ* in the mixing vessel for the last hour of steaming. At the conclusion of steaming, a flow of sterile air was introduced into the system via the backwash assembly. A positive pressure was necessary to prevent the system drawing in non-sterile air during cooling.

Once the system had cooled below 80ºC, SRO water was flushed through the system to cool the apparatus down to operating temperatures. Air was purged from the system by initiating a high flow rate (approx 20 l min^{-1}) through the biofilter. Flow was then set at 4 l min^{-1}, equivalent to a cross-filter flow velicity of 0.24 l min^{-1}. Temperature was maintained at 26ºC by adjusting the flow rate of tap water through the cooling circuit

(typically 1.5 l min^{-1}). If left unchecked, the heat produced by the pump raised the temperature of the system to 37ºC. The pH controller was activated and sterile 0.1M KOH (Fisher) pumped into the system as required to maintain the pH at 7.3. Once the temperature and pH were stable, the liquid level in the mixing vessel was lowered by opening the drain valve at the bottom of the filter to remove 1 l from the system. Sterile PYG medium was then added to the mixing vessel to achieve a final concentration of 0.1% in the system. The liquid level was then raised with SRO water to the level of the overflow in the mixing vessel (20 litres).

Mn was added as an acidified $MnSO_4$ solution and mixed with incoming SRO water at a point down-flow from the mixing vessel. Mn could not be added directly into the mixing vessel due to the possibility of chemically induced oxidation occurring around the site of KOH entry.

Inoculation of the large-scale biofiltration model was essentially the same as that described previously for the small-scale system, except that a larger 50ml inoculum of *Leptothrix discophora* SP-6 was used. Sampling was conducted typically once or twice a day, via one of the rubber septa along the length of the filter.

2.3. Manganese assay

The concentration of Mn in samples drawn from the biofilters were determined using a modification of the Formaldoxime method (HMSO 1997). All volumes were proportionally reduced to use a 7ml sample rather than the 100ml sample size described in the HMSO protocol. This method is based upon the colour change of a formaldoxime mixture which was proportional to the concentration of Mn in the sample. The colour

change reaction was stopped by the addition of ethylenediaminetetra-acetic acid disodium salt (EDTA) and hydroxyammonium chloride ($HO{\cdot}NH_3Cl$), at which point the adsorption at 450nm was measured on a spectro-photometer (CE 1020, Cecil Instruments Limited, Cambridge, UK). The inherent turbidity of the samples needed to be determined by a turbidity correction step to ascertain if any change in absorbance between samples was due to Mn concentration and not differences in turbidity. The turbidity correction step involved adding EDTA / $HO{\cdot}NH_3Cl$ to the sample before the formaldoxime mixture. Adding the components in this order prevented the reaction of the formaldoxime with Mn taking place and therefore any colour change from occurring. Absorbance values, minus the turbidity correction value, where compared to calibration curves constructed using standard solutions of known Mn concentration (Fluka) to yield the concentration of Mn in the biofilter sample.

2.4. Cell density measurements

While it is a standard microbiological technique to consider the optical density (OD) of a suspension of bacteria to directly correspond to the number of bacteria present, the flux between soluble manganese ions and insoluble manganic oxides expected to occur in these biofilter experiments may prevent this assumption from holding true. To elucidate this, serial dilutions of biofilter samples were prepared in sterile saline and examined by a Microcyte bench-top, flow cytometer (*Bio*DETECT AS, Oslo, Norway) to determine the number of cells ml^{-1}. The upper and lower particle size parameters for counting suspensions of *L. discophora* with the Microcyte were previously set by a skilled operator (C. Harding, Aber Instruments, Aberystwyth). When a stable peak was

displayed by the instrument (i.e. only minor fluctuations per second) data logging was initiated and the average of ten counts calculated. The Microcyte was flushed with SRO water between samples. These cell count data were transferred to a PC via a RS232C interface and then compared with the OD values obtained for the turbidity correction step of the Mn assay to determine if OD could be used as a reasonable indicator of cell density, particularly as there was an order of magnitude between the cell counts for the large and small scale biofilters.

3. Results

3.1. Control experiments

Control experiments, under aseptic conditions, in both the large- and small-scale biofilters showed no change in manganese concentration ([Mn]) during operation of the biofilter system, containing PYG medium. This showed that no chemical processes were causing Mn oxidation / accumulation. In addition, the optical density of the liquid in the system did not increase, indicating that the sterility of the system was maintained.

3.2. Small-scale biofilter experiments

The data from a typical bioaccumulation experiment in the small-scale filter system are shown in Figure 3. From an initial pre-filter (mixing vessel) [Mn] of 4739.92µg l^{-1}, [Mn] decreased to 119µg l^{-1} after 48 hours. The peak Mn bioaccumulation rate of 110.01µg l^{-1} h^{-1} occurred between 24 and 30 hours in this instance.

Between the same time points (24 and 30 hours) used to calculate the observed Mn bioaccumulation rate, the cross-filter drop in [Mn] (pre-filter minus post-filter [Mn])

was 221.53µg l^{-1}. Since the residence time was known, the number of times the contents of the mixing vessel pass through the filter in an hour was also known (the inverse of the residence time, giving 0.576 h^{-1}), enabling prediction of the Mn bioaccumulation rate from the mean cross-filter drop between these time-points to verify the results. The value of the predicted Mn bioaccumulation rate determined by the mean cross-filter decrease, equates to 127.60µg l^{-1} h^{-1}, within 14% of the observed Mn bioaccumulation rate.

During successive loading with Mn during a single experiment (Figure 4), the small-scale biofilter continued to retain Mn. The Mn bioaccumulation rate for subsequent Mn loads increased with time (Table 1) showing that the filter continued to mature.

The small-scale manganese bioaccumulation experiments where repeated (n=13) using the initial [Mn] as the experimental variable. These data were normalised (i.e. maximum value = 1) and collated to yield the initial [Mn] that gave the optimum maturation parameters (i.e. highest Mn bioaccumulation rate, highest optical density (cell density), highest increase in optical density (growth rate) and time of highest optical density (shortest maturation time).

3rd order polynomial trendlines through these data suggest that the optimum maturation initial [Mn] occurred between 2 000 and 3 000µg l^{-1} (Figure 6).

3.2. Large-scale biofilter experiments

The large-scale biofilter also accumulated Mn, mediated by *Leptothrix discophora*. In this instance (Figure 5), the [Mn] decreased from 5929.13 to 1928.62µg l^{-}

^{1} over a period of 144 hours. The Mn bioaccumulation rate between 17 and 144 hours was 30.51µg l^{-1} h^{-1}.

This showed that Mn bioaccumulation can occur in biofilters using filter packing media with much larger void spaces (25mm pebbles) than those typically used (sand) at water treatment sites. The Mn bioaccumulation rates were lower than those observed in the small-scale biofilter.

3.3. Cell density measurments

To determine if OD could be used as a direct estimate of cell density in the system, the OD values, obtained as the turbidity correction step of the Mn assay, were compared with flow-cytometric cell counts. These data showed good correlation (Figures 7a and 7b) - (Pearson's correlation; 0.998 and 0.991 respectively), showing that changes in optical density were directly proportional to cell density and not due to extraneous effects such as fluctuations in Mn solubility, temperature or pH. This also held true for measurements in the large-scale biofilter, operating with 0.1% PYG medium, although the OD values where relatively low (~10% of the small-scale filter) and the presence of suspended manganic oxides particles were thought to be more problematic.

4. Discussion

Manganese biofiltration was successfully modelled, in batch cultures, at two scales; that of the small-scale, laboratory (5 l) and the large-scale, pilot (20 l). Manganese bioaccumulation rates were generally higher in the small-scale filter than in the large-scale filter. This was probably due to differences in nutrient concentrations (the

large-scale filter operated at 0.1% PYG medium) and a reduced surface area for mass transfer to occur in the large-scale model, by the use of pebbles rather than sand as in the small-scale model. The high flow rates produced by the centrifugal pump may also have macerated planktonic cells as the liquid was constantly re-circulated through the pump via the flow control loop (figure 2) to achieve the desired flow rates whilst reducing pressure in the flow control system.

At a groundwater treatment site, any changes made to the operating parameters of a biofilter may cause a shift in the composition of the bacterial population within the filter biomass. This population shift could obscure (or magnify) the real effects of the parameter change on the organisms of importance, such as *Leptothrix discophora*, for biological Mn oxidation. A monoculture of *Leptothrix discophora* allowed direct comparisons to be made between different operating regimes, allowing the true effects of a parameter change to be observed.

The biological removal of Mn from water has previously been modelled by using the bacterium *Pedomicrobium manganicum* ACM 3067, which was isolated from an Mn oxidising biofilm within a water distribution system (Sly et al 1993). In this study, the bacteria were first immobilised onto magnetite particles, which were then incorporated into a continuous fluidised bed reactor. The authors showed from an initial [Mn] of 1 000µg l^{-1}, after 1 days operation at a residence time of 21 hours, the [Mn] fell to ~80µg l^{-1}.

The growth kinetics of *Leptothrix discophora* SP-6 observed in the small-scale biofilter revealed that the stationary phase was reached after approximately 30 hours (Figure 3). Liquid cultures of *Leptothrix discophora* SS-1 enter the stationary phase after

about 20 hours growth (Zhang et al 2002). In PYG liquid cultures, the growth rates of the two strains of *Leptothrix discophora* SS-1, and SP-6 are 0.7 h^{-1} and 0.5 h^{-1} respectively (Emerson 1992). This would suggest that the presence of the biofilter has a minimal effect upon the growth kinetics of the organsism compared to growth in 'typical' liquid cultures.

The data presented in Figure 3 is included as a typical example of a manganese bioaccumulation experiment (Hope 1999). A total of thirteen separate experiments were conducted using the small-scale biofiltration apparatus, the experimental variable being the initial [Mn]. The results of these experiments were collated (Figure 6). The initial [Mn] was plotted against the Mn bioaccumulation rate (µg l^{-1} h^{-1}), the maximum planktonic cell density (optical density units), the time of the maximum planktonic cell density (h) and the growth rate (h^{-1}). When these data were normalised (i.e. maximum value = 1) and viewed together with 3rd order polynomial trendlines. These data suggested that an initial [Mn] of between 2 000 and 3 000µg l^{-1} yielded the most rapid filter maturation time with the maximum cell density.

The optimum growth conditions for *Leptothrix discophora* were elucidated previously (Adams 1986), i.e. PYG medium buffered with HEPES at 25-26ºC, so nutrient concentration was not a parameter which we studied. By carrying out biofiltration experiments at optimum conditions it was possible to carry out a far greater number of experiments than those occurring in the field where maturation times are routinely measured in weeks, if not months.

It was important to establish that increasing optical density could be used as an indicator of bacterial growth and not increasing turbidity due to changes in the chemistry

of the filter system (i.e. increasing amounts of insoluble manganic oxides). Flow-cytometry was conducted using a bench-top flowcytometer, which has been shown to be a reliable method of monitoring cell number, viability, and apoptosis in mammalian (Harding 2000) and bacterial cell cultures (Rattanasomboon 1999). The cell counts derived by flow-cytometry were proportional to the optical density measurements taken from both small-scale and large-scale filter samples (Figures 7a and 7b), confirming that this association was not adversely affected by extraneous factors.

Constructing the large-scale biofilter introduced several fundamental problems that had to be overcome before the model could be used. The most important of these was maintaining flow across the biofilter section. An extension of the post-filter QVF tubing was made the highest point in the system in order to prevent gravitational drainage through the filter, which was predicted to occur under certain operating modes, i.e. reverse flow. The initial design of the large-scale filter used grade 8/16 sand as a filter packing medium, i.e similar to the small-scale version. However, during initial testing, the build-up of high pressure above the filter medium caused safety concerns. This problem would only have been exacerbated by biofilms of bacteria growing within the void spaces (Schwarz et al 2001; Goldgrabe et al 1993).

It was decided to use a filter packing medium with a larger void space, such as 25mm pebbles, to reduce the problem of pressure drop across the filter. We showed that Mn bioaccumulation could still occur with such large (relatively) void spaces and a reduced surface area. It was correctly assumed that the surface area would remain sufficient to provide a measurable bioaccumulation of Mn since the majority of the biological iron removal, for example, occurs in only the upper few centimetres of packing

sand (Ward 1994b). By not using sand, however, no difference in Mn concentration in the water could be measured through the length of the filter, because the removal rate from the relatively small surface area was not sufficient for detection.

In one instance, due to a malfunction of the pH controller, excess alkali entered the large-scale filter, chemically oxidising the entire mass of manganese in the system. However, the resulting insoluble manganic oxides were successfully filtered from the system, due to physical processes, by the pebbles in the biofilter whilst operating in the upwards flow mode. It may be assumed this was due to gravitational sedimentation of the oxides in regions of low hydrodynamic shear above individual pebbles. Backwashing the filter with water at 20 l min^{-1} and air at 4 min^{-1} dislodged the oxides from the medium. This phenomenon presents the possibility of using (bio)filters of a large particle size to remove suspended material from drinking waters. These filters would not become blocked with time; they would merely become saturated at which point they could be backwashed clean.

5. Conclusions

The collated data from the small-scale model suggests that the optimum initial Mn concentration is between 2 000 and 3 000µg l^{-1}. The relatively non-problematic scale up to the large-scale biofilter suggests that it would be possible to seed a new manganese biofilter with re-circultating, laboratory grown batch cultures of *Leptothrix discophora* SP-6. Data from the large-scale filter suggests that mature biofilms can bioaccumulate Mn upon filter packing media with a large void space, increasing the efficiency of the filter and reducing the need for backwashing. It is hoped that in combination, these

techniques would improve filter efficiency and reduce filter maturation times from months to days.

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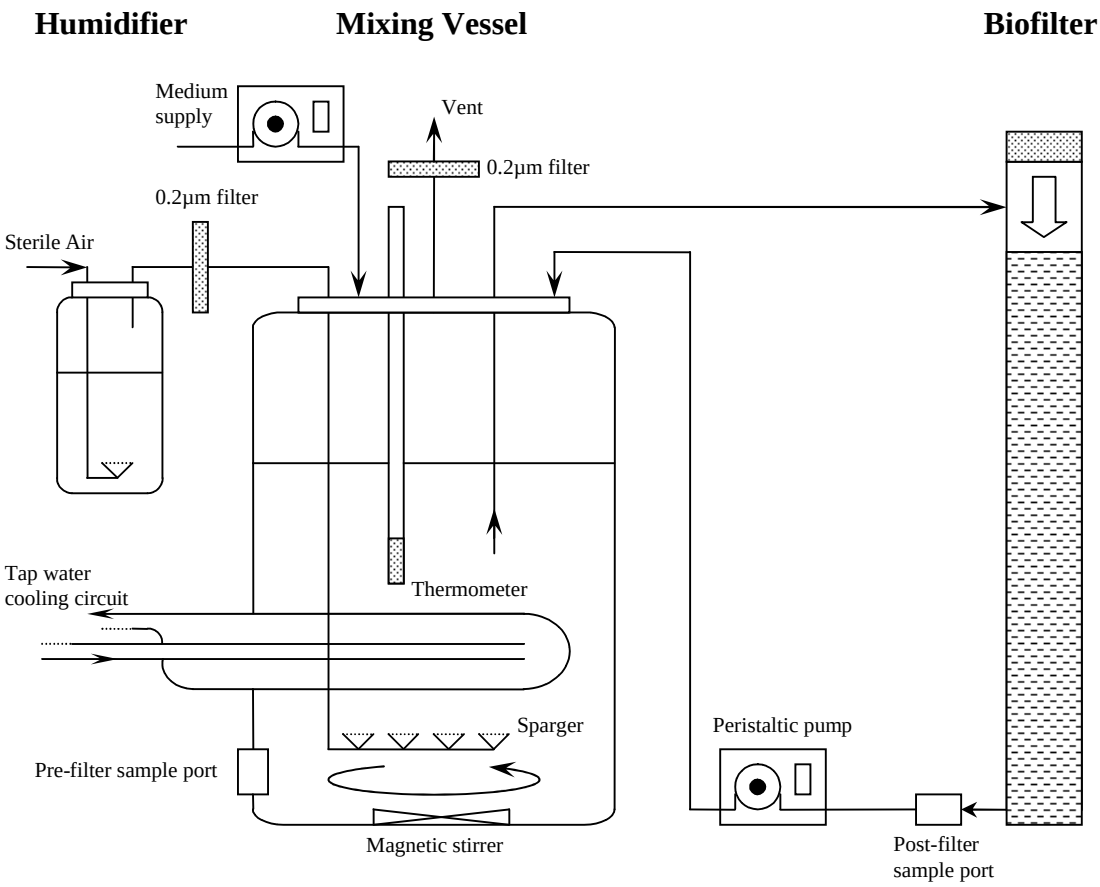


Figure 1. Schematic of the small-scale (5 litre) biofilter. The flow rate through the filter is controlled by the peristaltic pump, the temperature is controlled by the tap water flow rate through the cooling circuit.

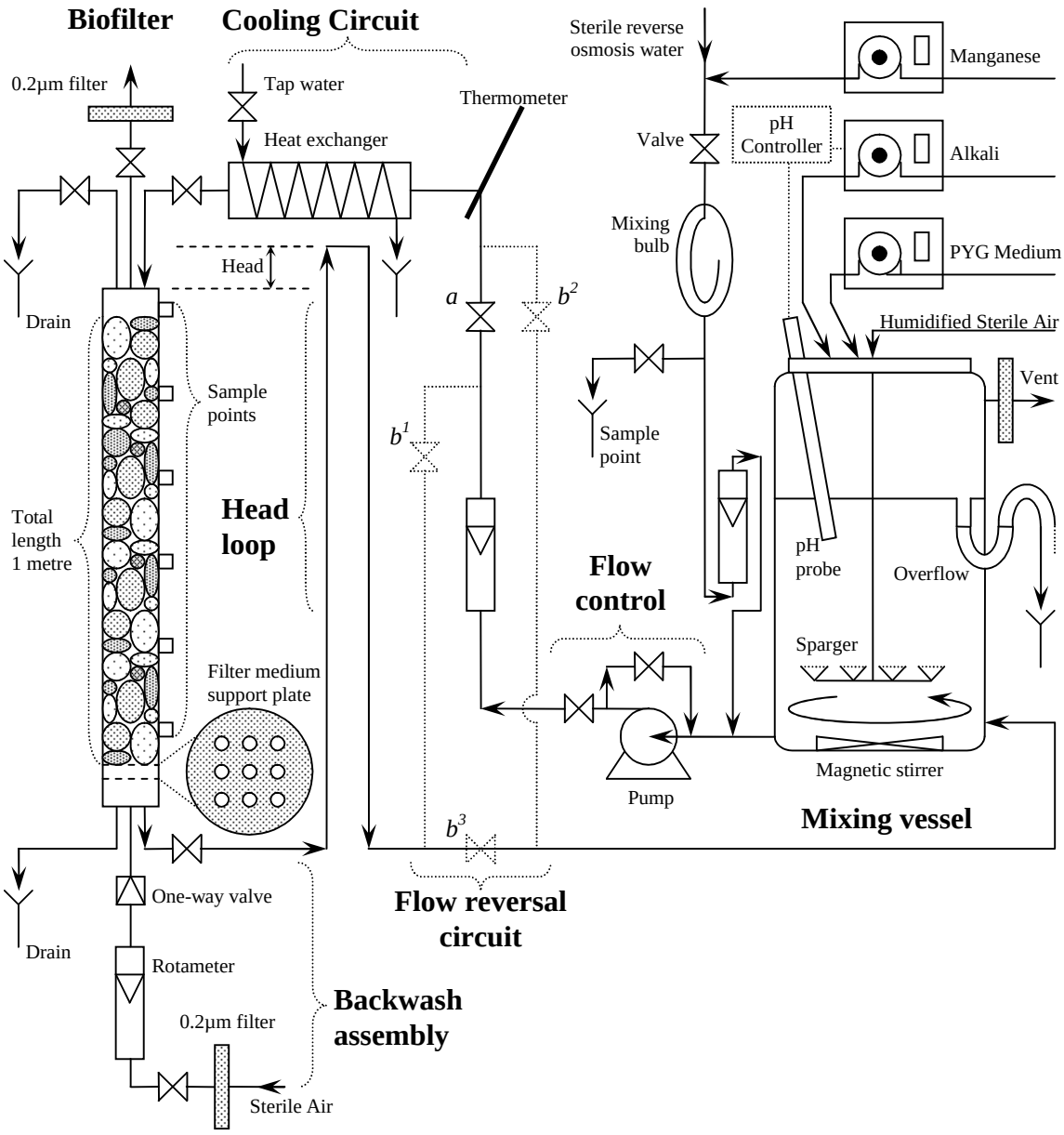


Figure 2. Schematic of the large-scale (20 litre) biofilter. Flow through the biofilter is controlled by the valve assembly shown in the diagram as 'flow control'. The 'flow reversal circuit' allows the direction of flow to be reversed by closing valves *a* and b^3; opening valves b^1 and b^2. Temperature is controlled by adjusting flow of tap water through the cooling circuit. The backwash assembly allows the biofilter to be cleaned with an air and water mixture at a high flow rate. The 'head loop' is the highest point in the entire system.

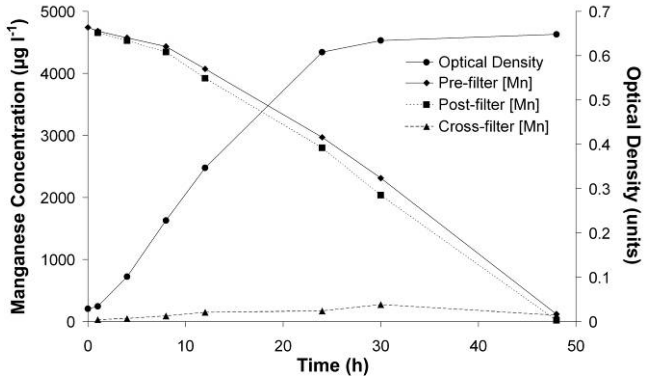


Figure 3. Bioaccumulation of Mn in the small-scale biofilter. Cross-filter [Mn] equals the difference between pre-filter and post-filter [Mn].

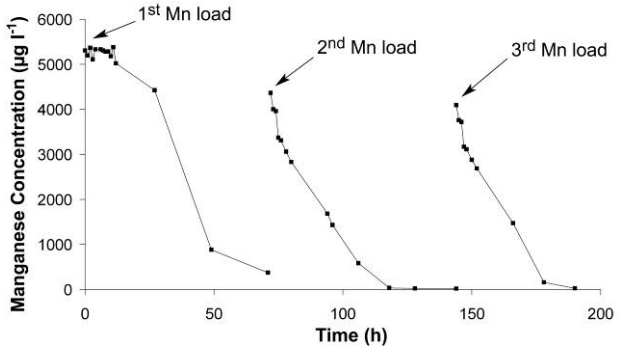


Figure 4. Bioaccumulation of Mn in the small-scale biofilter upon successive loading with Mn. 1st , 2nd and 3rd refer to three separate occasions where Mn was introduced into the system.

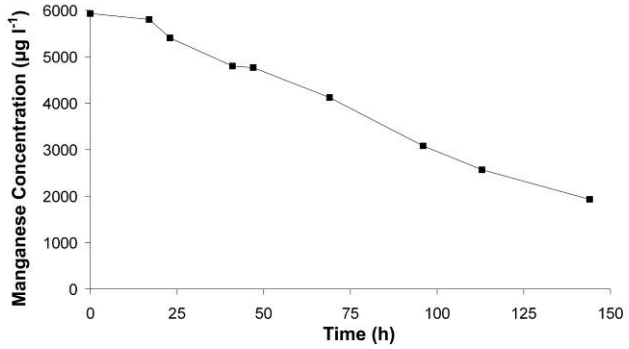


Figure 5. Bioaccumulation of Mn in the large-scale biofilter

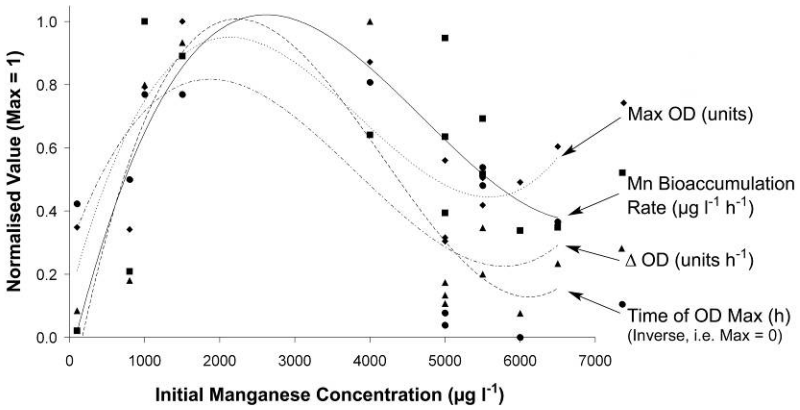
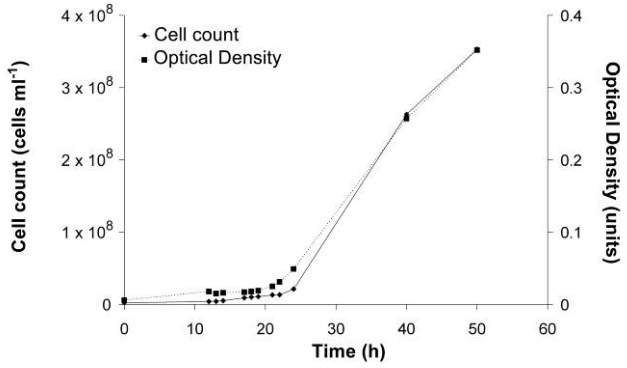


Figure 6. Collated data from all small-scale biofilter experiments. 3rd order polynomial plots of the initial Mn concentration vs normalised values (i.e. maximum value=1) of the optical density at the beginning of the stationary phase (◆; units), the maximum Mn bioaccumulation rate (■; µg l^{-1} h^{-1}), the rate of change in optical density during the growth phase (▲; units h^{-1}) and the time of the stationary phase OD (●; h). The normalised values for the time of the stationary phase are inverted (i.e. shortest maturation time = 1).

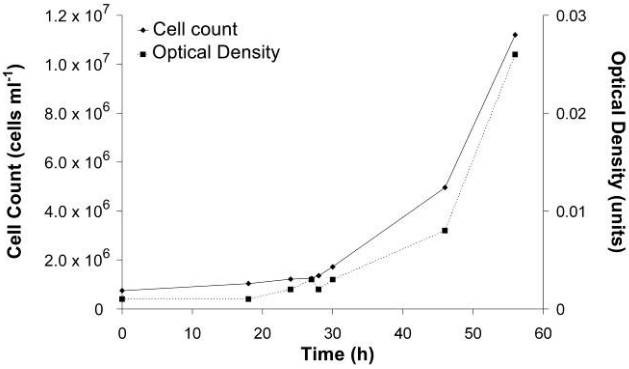
Table 1. Mn bioaccumulation rates for three successive Mn loads in the small-scale biofilter. 'Start Point' and 'End Point' refer to the time and [Mn] values used to calculate the Mn bioaccumulation rate (see figure 4).

	1st Mn Load		2nd Mn Load		3rd Mn Load	
	Time (h)	[Mn] (µg l^{-1})	Time (h)	[Mn] (µg l^{-1})	Time (h)	[Mn] (µg l^{-1})
Start Point	11	5377.2	72	4361.16	144	4093.20
End Point	71	372.24	106	583.13	166	1469.43
Mn bioaccumulation rate (µg l^{-1} h^{-1})	83.42		111.12		119.262	



a

Figure 7a. Comparison between optical density (◆) and cell counts (■) in the small-scale biofilter. Pearson's correlation: 0.998.



b

Figure 7b. Comparison between optical density (◆) and cell counts (■) in the large-scale biofilter. Pearson's correlation: 0.991.