ASSESMENT OF A NOVEL FILTER SYSTEM FOR RECIRCULATING AQUACULTURE

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Declaration

This thesis is a compilation of original research conducted by the candidate and has not been submitted for any other qualification. Information from the work of others (published and unpublished) has been duly acknowledged.

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Abstract

The aim of this project was to investigate the usage of manganese dioxide ore as a bio-filter media to remove metabolites in aquaculture closed system, and to determine whether manganese toxicity would at the same time represent a risk to fish.

Initial work investigated the physical properties of manganese dioxide and its chemical interaction with ammonia and nitrite in the absence of biological activity. Subsequently, two pilot-scale pressurised filters were installed in a commercial scale hatchery in order to compare the metabolite removal performance of manganese dioxide against silicate sand in the presence of biological activity commonly found in aquaculture conditions.

The investigation suggests that Mn medium is more reliable in converting ammonia to nitrate without producing a residual output of nitrite. The superior performance of Mn media compared with sand appears to be mainly related to the physical structure of the manganese ore. Furthermore, the Mn medium did not appear to be soluble in the ambient conditions normally found in aquaculture-closed system.

From the design point of view, due to the higher ammonia and nitrite removal rates, a shorter retention time and a lower volume of media are required in the case of manganese dioxide technology compared with sand media. As a result, it is much easier to size a biofilter with Mn media.

Manganese systems have a comparable total costs to conventional sand media, but using the Mn technology provides a more reliable control of toxic nitrite, thereby reducing risks of fish loss and hence with reduced expected production costs.

Glossary

BET	Brunauer-Emmett-Teller
BOD	Biological Oxygen Demand
BVs	Bed volumes
$CaSO_4$	Calcium sulphate
CO_2	Carbon dioxide
$CaCO_3$	Calcium carbonate
DD	Double distilled
EBCT	Empty bed contact time
GAC	Granular activated carbon
HCO_3^-	Bicarbonate
KCl	Potassium cloride
$NaHCO_3$	Sodium bicarbonate
NH_4^+	Ammonia ionised form
NH_3	Ammonia un-ionised form
NH_3-N	Ammonia nitrogen
NH_2OH	Hydroxylamine
NO_2^-	Nitrite
NO_3^-	Nitrate
$MgSO_4$	Magnisium sulphate
Mn	Manganese
$[Mn_{sol}]$	Concentration of soluble manganese
$[Mn_{tot}]$	Concentration of total manganese

MnO_2	Manganese dioxide
O_2	Oxygen
RASs	Recirculating aquaculture systems
SEM	Scanning electron microscopy
SG	Sintered glass
SS	Silicate sand
SSA	Specific surface area
TAN	Total ammonia nitrogen
TKN	Total Potassium Nitrogen
TSS	Total suspended solid
UMS	Universal Mineral Supplies Ltd
VR	Volcanic rock

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Chapter 1
Introduction

1.1 The potential for development of recirculation systems in aquaculture

It is generally accepted that output from the world's fisheries is unlikely to increase significantly and that expansion of the aquaculture sector will almost certainly be required to address the problem of the projected shortfalls in supply (Chamberlain and Rosenthal, 1995). The aquaculture industry is the world's fastest growing food-producing sector, with an annual growth rate of almost 10% between 1984 and 1995, compared with 3% for livestock meat and 1.6% for capture fisheries production (FAO, 1997).

Currently there is still an expansion of conventional farming systems based on low intensity production systems with limited or moderate control over environmental conditions. However, in many countries the aquaculture sector is evolving towards high-density, and high productivity systems, which require various degrees of control over the culture conditions (Rosenthal, 1993; Muir, 1998). Dependent on a wide range of environments, production systems and species targeted, intensive aquaculture ranges from limited to complete control over the culture conditions. Typically it is possible to distinguish between semi-intensive and intensive production systems that exchange large volumes of water to maintain adequate dissolved oxygen and water

quality in the culture, and production methods with zero or limited water exchange that are based on water re-use.

There are two kinds of approach in water re-use systems, ecological and mechanical (Muir, 1995). The ecological approach re-use the water by natural nutrient recycling, for example, a pond production system, which is never drained and can be harvested by seining and restocking the next season. In this case a rather large area is required to keep the business economically feasible because the stocking density is relatively low. In contrast, the mechanical approach treats the wastewater in a separate treatment system and returns the treated water to the rearing tanks. By using various unit processes to recycle and reuse culture water, recirculating aquaculture systems (RASs) can provide complete control over the culture environment, supporting high densities of fish under intensive feeding conditions. Summerfelt *et al.* (2000) differentiate between RASs that utilize a partial flow-through of water, resulting in one or more system exchanges daily and closed systems, defined as exchanging less than 10% of the system volume daily.

RASs provide an alternative approach to semi-intensive and intensive production systems that exchange large volumes of water, thus offering several advantages that are described below:

1) Without the water resource requirements and geographic restrictions of traditional aquaculture methods, RASs can be used at inland locations away from sensitive coastal areas, where multiple-use conflicts exist. This is of particular importance in

industrialised countries where extensive methods are generally not economically feasible because the cost of land use is extremely high.

2) In intensive systems, water is exchanged in order to maintain acceptable water quality because of the perceived benefits of preventing the accumulation of toxic metabolites. However effluent may contain high concentrations of organic matter and inorganic nutrients that can adversely affect the quality of receiving waters. In addition, water can serve as a transport medium for the introduction of non-native species into the wild where indigenous populations exist (Spotte, 1979). By using RASs, in which water requirements are reduced significantly, these types of risk can be minimized considerably.

3) Biosecurity in aquaculture is the protection of fish or shellfish from infectious (viral, bacterial, fungal or parasitic) agents. The primary goal of a biosecurity program in aquaculture is to prevent the introduction of any infectious organism into an aquaculture facility. Among the numerous potential sources of entry for an infectious agent into an aquaculture facility, pathogens can enter and infect cultivated animals via untreated intake water. Once established, pathogens can spread via untreated effluent discharged into the surrounding environment, which serves as a common water source for nearby farms. This makes water exchange a risky management option for aquaculture producers unless both influent and effluent streams are disinfected. However, the cost of disinfecting large volumes of water is very high. In recirculation systems water requirements are reduced significantly, thereby mitigating the risk for pathogen introduction into the culture environment through disinfection methods.

4) In both traditional aquaculture methods (ponds, flow-through system farms, sea cages, etc.) and RASs the amount of waste produced is in direct proportion to the quantity of feed used to feed the fish. Because the concentration of the wastewater effluent is directly related to the volume of water discharge, the most dilute wastewater effluents tend to result from conventional farming systems, while the most concentrated effluents are generated from RASs (Chen *et al.*, 1997; Twaroska *et al.*, 1997). As a result, the concentrated wastewater originating from RASs (Timmons and Losordo, 1994) can be more efficiently treated compared with effluent discharge from traditional aquaculture. RASs effluents can be de-watered or alternatively after full treatment using both aerobic and anaerobic methods, will commonly meet discharge requirements to waterways.

5) In comparison to conventional flow-through systems, RASs can reduce production costs mainly because much less energy is required for heating, and survival rates as well as the growth rates of the cultured animal can be much higher. This is particularly true in temperate climates, where sufficient quantities of warm water are not available all year round. In this context, depending on a range of environments and species targeted, the introduction of recirculation systems has the potential to speed up the development of reliable and cost effective production systems (Blancheton, 2000; Kirkup *et al.*, 2000).

6) By reducing the water requirements and controlling the environment, RASs have the advantage of allowing for year-round production. The production systems can be located reasonably close to the market, thus reducing transportation costs, stress and

moralities incurred during transportation. Thus, as a result recirculating aquaculture systems can be suited for serving live fish markets, especially in places where live fish command a premium price (Kirkup *et al.*, 2000).

However, RASs do have some disadvantages. Capital and operating costs as well as presumed unreliability problems are normally much higher for the RASs than that of traditional aquaculture methods (Nijhof, 1995). The economics of intensive recycle systems for the rearing of a variety of aquaculture species have been assessed by a number of researchers (Muir 1981; Losordo and Westerman 1991; O'Rourke 1991; Gempasaw *et al.* 1993). It has been suggested that in order to raise a crop of fish to compete with traditional aquaculture methods, recirculating systems must be capable of producing fish at a comparable expense, and that this first requires the construction of lower-cost systems (Chen *et al.*, 1994; Timmons and Aho, 1998). In this context one of the key issues is the development of new and innovative technologies for RAS design. Today aquaculture engineers' efforts focus on improving water quality treatment systems, improving monitoring and data assessment, and reducing RAS component costs (Weeks and Westers, 2002). In addition, various responses to the problem of price competition within the aquaculture industry have been considered:

a) A strategy to minimize costs has involved the development of systems with less expensive technologies and design. However, these types of system can only sustain water quality for the culture of less demanding species of fish, which generally suffer from relatively low value. An alternative strategy has considered the culture of a species of fish that hold a high value. In this case, the design of these systems has

utilized methods for minimizing equipment costs and operational expenses without compromising the quality of the system engineering design (Van Gorder, 2002).

b) For a given facility, total production costs can be reduced by increasing the production rate, which in turn can be achieved by either increasing the stocking density or the growth rate. However, in increasing stocking densities there are physiological as well as engineering and physical limitations that limit the culture intensity. As a result, high stocking density can negatively affect growth rates in many species of fish, although the responses vary (Kirkup *et al.*, 2000). Regardless of the actual effects of high stocking densities, increased concern for animal welfare may also limit the intensity to which producers may be permitted to operate.

c) It is generally believed that economies of scale exist for recycle systems as they scale up in size, because larger systems spread operating and capital costs over a greater level of production. Wade *et al.* (1996) demonstrated that economies of scale relationships exist for cold-water intensive recycle systems designed to raise rainbow trout. Based on a discounted cash flow analysis (O'Rourke, 1991), the profitability of a given system was determined by four main factors, capital costs, operating costs, annual revenues, and the required level of return or opportunity cost of capital for the project. Economies of scale have also been recently confirmed by several authors (Van Gorder, 2002; Wilton, 2002; Beckman *et al.*, 2002), who have presented commercial recirculation systems with an annual output of 50 – 100 tonnes. These studies seem to confirm that the trend is towards larger RASs, showing that the economy of scale is one of the best possible solutions to the problem of price competition within the aquaculture industry. Nevertheless, this does clash with a

higher risk to spread infectious agents to a larger fish stock; thus big RASs should be modularized in replicate systems to reduce pathogen transfer risk.

It is now widely acknowledged that in the most developed regions of the world the aquaculture industry is coming under greater environmental pressure, especially in terms of permit constraints on water supply and discharges. At the same time market competitiveness and the potential for economies of scale is increasing the commercial pressure for larger intensive aquaculture facilities (Timmons *et al.*, 1998; Muir, 1998). In this context, as economic issues are resolved, recirculating aquaculture systems will become more widely used in the industry, able to satisfy a higher production efficiency and especially to meet the environmental regulatory concerns threatening aquaculture.

1.2 The technological background of recirculation systems in aquaculture

In order to sustain intensive aquaculture, closed systems require the integration of carefully designed hardware and technologies (Losordo and Westerman, 1991) with specifically co-developed management techniques (Wheaton, 2002). The RAS is a complex environment, involving physical, physiochemical, chemical and biological treatment methods, thus a specific configuration accepted as the 'state of art' in systems design does not currently exist. Nevertheless, the critical requirements, and the process engineering options available for most effectively achieving the necessary water quality control in closed systems have been clearly identified and demonstrated (Timmons and Losordo, 1994).

The application of recirculation technology to fish culture has been suggested since the early 1970s (Speece, 1973; Forster, 1974; Liao and Mayo, 1974). Liao and Mayo (1974) highlighted the most important treatments required to reconditioning RAS water including: (a) Removal of metabolites (faces, ammonia, carbon dioxide and organic solids); (b) Effective aeration/oxygenation; (c) pH control. The same principles are still valid today, where the following physical, chemical and biological treatment methods are commonly adopted in RASs (Timmons and Losordo, 1994):

1) Mechanical aeration and chemical oxygenation;
2) CO_2 desorption by air stripping;
3) Ammonia converted to nitrates with autotrophic species of bacteria (nitrifying bacteria) in filter;
4) Nitrate converted to nitrogen with anaerobic species of bacteria (denitrifying bacteria) in filter;
5) pH dosing by chemicals;
6) Suspended solids removal by mechanical filters;
7) Organic nitrogen conversion to inorganic state by heterotrophic species of bacteria in filter;
8) Pathogen control with ultra-violet irradiation and ozone treatment;

1.2.1 Removal of metabolites

The principal excretory product of fish metabolism is nitrogen as ammonia, which exists dependent on a pH and temperature –governed equilibrium between a relatively

innocuous ionised form (NH_4^+) and a toxic un-ionised form (NH_3). Acute toxic levels for NH_3 vary between fish species (Kruner and Rosenthal, 1983), and therefore knowledge of the maximum safe levels of NH_3 that result in acceptable growth and health of fish is required for efficient RAS production. The removal of ammonia via biological oxidation is used in RASs because it is cheaper and simpler compared with physical-chemical methods (Muir, 1982; Metcalf and Eddy, 1991). The latter also usually require special operator skills because under certain conditions they can be harmful to fish (Liao and Mayo, 1974).

Nitrification is the biological process in which the ammonia nitrogen is removed via transformation to nitrite (NO_2^-) and nitrate (NO_3^-). The nitrifying bacteria are attached to fixed surface (filter media) contained within the filter structure (Spotte, 1979). Nitrite is toxic to vertebrates including fish and the principal effect is the conversion of haemoglobin to methaemoglobin, which is incapable of oxygen transport. Nitrite toxicity is strongly alleviated by chloride and the concentration ratio of these ions is of great importance in assessing toxicity (Perrone and Meade, 1977; Bath and Eddy, 1980; Russo *et al.*, 1981; Eddy *et al.*, 1982; Eddy and Williams, 1987).

The nitrate ion, as a final product of nitrification process, is considered to be relatively harmless to several fish species over a wide range of concentrations (Muir, 1982). However nitrate accumulation in RAS ought to be prevented to avoid pH values decreasing continuously (Sharma and Ahlert, 1977) and to meet environmental and public health regulatory discharges existing in many countries. The maximum levels of nitrate allowed in the effluent water differ from country to country and are as low

as 11.6 mg L^{-1} NO_3-N in Europe according to European Community directive (Van Rijn and Rivera, 1990). It is not uncommon to find in RASs nitrate concentrations of up to 200 – 300 mg L^{-1} NO_3-N (Owers, Personal communication); anaerobic biological filters are often integrated in the RASs design to control nitrate.

The removal of waste solids is critical in the operation of recirculating aquaculture systems (Tetzlaff and Heidinger, 1990). Suspended solids can build up in recirculating systems to the level where the entire system will fail (Tetzlaff, 1991). The accumulation of solid wastes, mainly in the form of faecal matter and uneaten food, is detrimental to fish health. The removal of suspended solid in aquacultural production systems is generally carried out by mechanical treatments. Among the different methods, drum screens are used frequently in recirculating aquaculture systems for solid removal (Mortensen, 2002). These are rotary screens, which are usually run by gravity where an intermittent backwash prevents the rotary screen from plugging. In relation to suspended solids concentration and their particle size, typical RASs applications use filter-openings from 60 – 100 micron, which are able to develop a high capacity up to 1,500 L s^{-1} in one unit (Wheaton, 2002; Mortensen, 2002). However, depending on the characteristics of the production system and species targeted, other mechanical solids removal systems can be used such as centrifuges and hydrocyclones, sand filters and bead filters. In addition, when very clear and transparent water is needed, the removal of the finest residual suspended solids is often carried out by chemical methods including foam fractionation and ozonation (Chen *et al.*, 1994).

The degradation of the dissolved fraction of organic waste in closed systems, originating from fish excretion and excessive feed, is mainly carried out by biological oxidation inside the filter (Otte and Rosenthal, 1979). Heterotrophic species of bacteria, sharing the filter space with nitrifying bacteria, mineralise organic compounds to simple compounds such as ammonia. However, the removal of residual low-biodegradable substances is more often carried out by chemical methods including adsorption (Spotte, 1979; Wheaton, 2002), foam fractionation (Timmons, 1994) and ozonation (Summerfelt *et al.*, 1997).

Closed systems, supporting high fish loading rates with the addition of large amounts of pure oxygen, can provide insufficient gas exchange to strip the quantities of carbon dioxide produced. Carbon dioxide can produce adverse effects over a wide range of concentrations depending on the fish species cultured (Muir, 1982). For the most effective carbon dioxide stripping, these systems will require aeration units designed to contact large volumes of air (as much as 10 volumes of air per volume of solution) with water (Grace and Piedrahita, 1993; 1994; Summerfelt *et al.*, 2000a). Therefore, aeration systems and sub-surface aerators, which move air bubbles through water, are generally less effective at stripping carbon dioxide than surface aerators and packed column aerators that rely on moving water droplets through air (Colt and Orwicz, 1991).

1.2.2 Aeration/Oxygenation

In addition to the mechanical aeration used in water reconditioning in the early 1970s (Liao and Mayo, 1974), the application of closed systems of higher stocking density

(Summerfelt *et al.*, 2002), requires the addition of oxygen to maintain adequate dissolved oxygen in the culture. Moreover, aerobic bacteria in the filter also demand large amounts of oxygen for their oxidation activities: for example each mass unit of ammonia oxidized to nitrate requires 4.18 mass units of oxygen (Timmons and Losordo, 1994). There are different methods of adding oxygen in systems, either by producing it on site, or by off-site generation (usually liquid) and bulk transporting it to the aquaculture facility. Monita (2002) discussed various methods including liquid oxygen supply and on-site generation using either PSA (Pressure Swing Adsorption) or VSA (Vacuum Swing Adsorption) systems. Liquid oxygen has high operating costs and a considerable amount of work is required for the management of the liquid supply. VSA system produces oxygen at very low pressure and eliminates any risk associated with high pressure PSA system. Overall, Monita (2002) concluded that VSA system offers a more favorable option with lower cost compared to either a liquid oxygen supply system or a PSA system.

1.2.3 pH control

The pH of closed systems is controlled by effective carbon dioxide stripping, but more importantly by the continuous addition of chemicals to restore the original alkalinity of the water supply, that is reduced during the nitrification process: each mass unit of ammonia oxidized to nitrate requires 7.14 mass unit of alkalinity (Timmons and Losordo, 1994). A range of mineral supplements has been suggested by Spotte (1979) to maintain steady pH conditions in closed systems.

1.2.4 Process flow

Gradually the RAS process flow system has been changed from a simple 'ring' system, in which each process is controlled by the process step prior to it (Muir, 1981), towards a more complex multi-loops 'radial' system (Rosenthal, 1981a; Muir, 1982). The multi-loop system allows for optimisation of treatment efficiency and control over individual processes (Rosenthal, 1993). In 'radial' design the 'main stream' flow generally treats all the water flowing through the system while the 'side stream' flow treats only a variable proportion of all the flow, depending on several factors such as system design, production criteria and water quality requirements. The 'main stream' generally incorporates a mechanical filter (drum screens) and biological filter, while the 'side stream' water treatment includes a more efficient mechanical filter (sand filter, bead filter), followed by various chemical methods including adsorption, foam fractionation, ozonation and UV irradiation. In summary, the 'main stream' treatments are responsible for the bulk removal of metabolites whereas the 'side stream' water treatments produced high purity water, particularly with non-detectable levels of fine suspended solids and pathogens.

1.3 Biological filter principles in aquaculture

Various researchers have reached the conclusion that a 'steady-state' of the culture conditions is not possible in high-density fish culture (Honer, *et al.*, 1985; Poxton and Allouse, 1987). In recirculation fish culture systems, continued equilibrium between rates of metabolite accumulation and rates of biodegradation in treatment units is impossible to achieve (Rosenthal, 1993). Moreover, substantial fluctuations of water

quality parameters (metabolites, pH and Oxygen levels) can occur under intensive culture conditions as a result of the feeding methods employed, feeding level and fluctuations in feed utilization (Rosenthal *et al.*, 1980; Kamstra *et al.*, 1998). Under these conditions operating levels will affect efficiency of treatment of individual parameters, particularly if treatment is concentration-dependent, as are most of the biological methods. To complicate the matter, physical and chemical treatments are generally able to act instantaneously while biological treatments, because of the time required for cell growth, are able to respond only with an appreciable delay (Rosenthal, 1981a; Muir, 1982). As a result of the characteristics of biological treatment, fluctuations of the culture conditions may reach critical levels for ammonium and nitrite several times a day (Rosenthal et al., 1980).

The biological treatments adopted in RASs are complex processes to control. This is also in accordance with the general trend in water treatment, where such processes are unlikely to fail, but more likely to under-perform (Muir, 1982). Nitrification is a key process in the water treatment of a recirculation system (Speece, 1973), and the accumulation of ammonia, the principal nitrogenous excretory waste of cultured fish and invertebrates, can limit the carrying capacity of aquaculture systems employing water re-use. Furthermore, nitrifying bacteria in the biological treatment oxidise ammonia via nitrite to nitrate, but where the oxidation of ammonia is incomplete, higher concentrations of nitrite compared to ammonia can occur (Otte and Rosenthal, 1979; Poxton *et al.*, 1981; Bovendeur *et al.*, 1987; van Rijn and Riviera, 1990), which can even impose further limitations on the use of nitrification in RASs.

The term biological treatment includes all treatments that utilize biological organisms to remove chemicals, organic and inorganic compounds from water. The principal applications of this process in aquaculture are:

- The removal of the carbonaceous organic matter, usually measured as carbonaceous BOD (Biological Oxygen Demand, see also Appendix B);
- Nitrification;
- Denitrification;

In this chapter, the emphasis will be on nitrification, in which toxic ammonia nitrogen (NH_3-N) is removed via transformation to nitrite (NO_2^-) and nitrate (NO_3^-). This section will first discuss aspects of microbial metabolism in biological treatments. Secondly, the nitrification process in treatment systems will be presented, including the main parameters affecting removal rates. Finally, common nitrification filter configurations found in RASs will be described and the major variables effecting filter operation will be discussed.

1.3.1 Microbial metabolism principles

Bacteria can be classified based on the carbon and energy source, usually referred to as substrates, used to carry out reproduction and basic function. The most common sources of cell carbon are organic matter used by *heterotrophs* and inorganic carbon (i.e. $CO_{2\,(aq)}$ or HCO_3^-) used by *autotrophs*. The energy needed for cell synthesis may be supplied by light or by a chemical oxidation reaction. Because of the filter configuration normally used in aquaculture, in which light is very limited or not

existent, the removal of carbonaceous organic matter and ammonia nitrogen are thought mainly to involve *Chemoheterotrophic* and *Chemoautotrophs* bacteria.

Chemoheterotrophic bacteria can be further classified accordingly to their metabolism type and their requirement for molecular oxygen. Organisms that generate energy by enzyme-mediated electron transport from an electron donor to an external electron acceptor are said to have a *respiratory metabolism*. In contrast, *fermentative metabolism* does not involve the participation of an external electron acceptor. When molecular oxygen is used as the electron acceptor in respiratory metabolism, the process is known as *aerobic respiration*. In particular *obligate aerobic* refers to organisms able to function only if there is a supply of molecular oxygen. In the absence of molecular oxygen, oxidized inorganic compounds such as nitrate and nitrite can function as electron acceptors from some respiratory organisms, and those which can do so are defined as *facultative aerobic* bacteria. It is also possible to distinguish between *obligately anaerobic* and *facultative anaerobic* bacteria. The first generate energy by fermentation and are able to survive only in an environment in absence of oxygen, while *facultative anaerobes* can grow either in the presence or absence of molecular oxygen.

The biological community in filtration treatments includes *Chemoheterotrophic* and *Chemoautotrophic* bacteria. *Chemoheterotrophs* can be aerobic, anaerobic, and facultative, although there is evidence suggesting that facultative bacteria are predominant (Metcalf and Eddy, 1991).

Chemoheterotrophic bacteria are responsible for 'ammonification', that is, the decomposition of complex organic compounds via transformation to simpler organic compounds and then to ammonia (Anthonisen *et al.*, 1976). Bacteria of the genera *Achromobacter, Flavobacterium, Pseudomonas and Alcaligenes,* are commonly associated with this biological process (Metcalf and Eddy, 1991). *Chemoautotrophs* are usually aerobic, using dioxygen gas (O_2) as the final or terminal electron acceptor. Although several genera of *Chemoautotrophic* nitrifying bacteria have been identified, the genera *Nitrosomonas* and *Nitrobacter* are responsible for most of the naturally occurring nitrification (Hagopian and Riley, 1998). These bacteria are short rods (0.8 x 1 – 2 μm), typically non-motile, and ubiquitous in soils (Watson, 1971; Bock *et al.*, 1989). *Nitrosomonas* and *Nitrobacter* are characterized by the ability to utilize respectively ammonia nitrogen and nitrite as a source of electrons for the mobilization of inorganic carbon into biomass.

Basic chemical conversion carried out by bacteria:

In order to convert carbonaceous organic matter (COHNS) in new bacterial cells ($C_5H_7NO_2$), *Chemoheterotrophic* bacteria carry out endogenous respiration, in general accordance with the stoichiometry shown in Eq. 1 (Metcalf & Eddy, 1991):

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + 2H_2O + NH_3 + \text{energy} \qquad (1)$$

In contrast, *Chemoautotrophic* nitrifying bacteria carry out aerobic respiration in general accordance with the stoichiometry shown in equations 2 and 3 (Hagopian and Riley, 1998):

$$NH_3 + 1.5\ O_2 \rightarrow NO_2^- + H_2O + H^+ + 84\ \text{Kcal mol-1} \quad (2)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- + 17.8\ \text{Kcal mol-1} \quad (3)$$

The first exogenous reaction releases more than four times as much energy as the second (Gibbs and Schiff, 1960). The first reaction also produces hydrogen ions and as a result lowers the pH.

However, equation 2 represents a simplification of the true catabolic process of ammonia-oxidizing bacteria. It is now well accepted that hydroxylamine formation is an intermediate step in the oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (Hagopian and Riley, 1998). The process begins at the inside of the cytoplasmic membrane when ammonia is oxidized to hydroxylamine as shown in equation 4 (Painter, 1970; Hollocher *et al.*, 1981):

$$NH_3 + 0.5\ O_2 \rightarrow NH_2OH + 0.7\ \text{Kcal mol-1} \quad (4)$$

Hopper (1989) suggests that internal energy must be invested in order to activate the reaction shown in equation 4. As a result the cell derives little energy for the conversion of ammonia to hydroxylamine. This compound is then transported to the cell surface (periplasm) and converted to nitrite (equation 5):

$$NH_2OH + 0.5\ O_2 \rightarrow NO_2^- + 3H^+ + 83.3\ \text{Kcal mol-1} \quad (5)$$

This indicates that the net increase in acidity from nitrification is entirely traced to the conversion of hydroxylamine to nitrite. Moreover, Bock *et al.* (1991) and Frijlink *et al.* (1992) concluded that, although toxic at low concentration, hydroxylamine can serve directly as a substrate for ammonia-oxidizing bacteria.

Bacteria cell growth:

Chemoheterotrophic bacteria carry out the conversion of carbonaceous organic matter (COHNS) in to new bacterial cells ($C_5H_7NO_2$) and a range of stable end products in general accordance with equation 6, which describes cell synthesis and oxidation (Metcalf & Eddy, 1991):

$$COHNS + O_2 + \text{nutrients} \rightarrow CO_2 + NH_3 + C_5H_7NO_2 + \text{other end products} \quad \textbf{(6)}$$

Generally, nitrifying bacteria cell growth and ammonia oxidation to nitrate can be described as follows (Water Pollution Control Federation, 1983; Gujer and Boller, 1986):

$$NH_4^+ + 1.83\,O_2 + 1.98\,HCO_3^- \rightarrow$$

$$0.021\,C_5H_7NO_2 + 0.98\,NO_3^- + 1.041\,H_2O + 1.88\,H_2CO_3^- \quad (7)$$

From equation 7 it is possible to determine basic requirements and cell biomass production for the nitrifying bacteria: for every gram of NH_4^+-N oxidized to NO_3^--N, 4.18 g of O_2 and 7.14 g of alkalinity (as $CaCO_3$) are used and 8.59 g of carbonic acid and 0.17 g of cells are produced (Timmons and Losordo, 1994).

Equation 7 can be alternatively expressed by equations 8 and 9, where ammonia and nitrite oxidation and cell synthesis are carried out by two phylogenetically separate groups of *Chemoautotrophs* bacteria (Haug and McCarty, 1972):

Nitrosomonas

$$55\ NH_4^+ + 5CO_2 + 76O_2 \rightarrow$$

$$C_5H_7NO_2 + 54\ NO_2^- + 52H_2O + 109H^+ \qquad (8)$$

Nitrobacter

$$400\ NO_2^- + 5\ CO_2 + NH_4^+ + 195\ O_2 + 2\ H_2O \rightarrow$$

$$C_5H_7NO_2 + 400\ NO_3^- + H^+ \qquad (9)$$

From equations 8 and 9 it is possible to calculate the cell yield, which is defined as the measure of the mass of cells produced per unit mass of ammonia converted to nitrate (Timmons and Losordo, 1994). From the reaction stoichiometry, *Nitrosomonas* have a higher cell yield (0.147) compared with that of *Nitrobacter* (0.02) and heterotrophic bacteria usually have a higher cell yield (0.6) compared to nitrifying bacteria (Metcalf and Eddy, 1991; Figueroa and Silverstein, 1992; Golz *et al.*, 1996). Hagopian and Riley (1998) highlight that the maximum specific growth rate of nitrifying bacteria is uncommonly slow. Watson (1971) and Bock *et al.* (1989) suggest a doubling time for nitrifying bacteria of 7 – 8 h under ideal conditions. However, Shilo and Rimon (1982) measured a doubling time of 26 h for ammonia oxidizers and 60 h for nitrite oxidizers present in fishponds. Autotrophic doubling time is more than an order of magnitude slower than that of aerobic heterotrophs (Watson, 1971; Bock *et al.*, 1989),

as autotrophic transformation of carbon dioxide to cell tissue is a reductive process that requires a net input of energy. Therefore autotrophic organisms must use more of their energy for synthesis compared with heterotrophs, resulting in generally lower growth rates (Hagopian and Riley, 1998).

Growth and nitrification rates depend strongly on substrate concentration. In a mixed culture under a single limiting-substrate condition, the steady-state kinetics of substrate removal is usually described by the Monod-type expression (Srna and Baggaley, 1975; Rittmann and McCarty, 1980; Drtil *et al.*, 1993):

$$R = \upsilon_{max} (X / Y_S) (S / K_S + S) \qquad (10)$$

Where

R = substrate removal rate (g m^{-3} d^{-1}),

υ_{max} = specific growth rate (d^{-1}),

X = bacterial mass concentration (g cell m^{-3}),

Y_S = yield of bacterial mass per unit of substrate used (g cell g^{-1} substrate),

S = limiting substrate concentration (g m^{-3}),

K_S = half saturation constant (g m^{-3}).

Growth and nitrification rates also depend on temperature and pH. Generally, the optimum pH for bacteria growth lies between 6.5 and 7.5 (Metcalf & Eddy, 1991; Timmons and Losordo, 1994). Nitrifying bacteria can also adapt to a wide range of temperatures. In a suspended culture, biological reaction rates increase with rising temperature until an optimal temperature is reached. Above the optimal temperature,

enzymatic proteins denature and the rates decrease (Sawyer *et al.*, 1994). Wortman (1990), studying the effects of the temperature from 7 to 35 °C on nitrification in a biodrum, found a linear relationship between nitrification rate and temperature. As a very general biological rule growth rate of bacteria doubles with approximately every 10 °C increase in temperature until the optimal temperature is reached (Metcalf & Eddy, 1991). Temperature effects on biological reaction rate can be generally described by the van't Hoff-Arrhenius equation (Tchobanoglous and Burton, 1991):

$$\upsilon = \upsilon_{20}\ \theta^{T-20} \qquad (11)$$

Where

υ= the rate coefficient (d^{-1}),

υ_{20} =the value of υ at 20°C (d^{-1}),

θ = temperature coefficient (dimensionless),

T = temperature (°C).

1.3.2 Nitrification in treatment systems

It is possible to distinguish between aerobic suspended-growth biological processes, mainly used for the removal of carbonaceous organic matter and aerobic attached-growth biological processes used for nitrification. Suspended-growth processes use the bacteria floc itself as the substrate; while in the attached-growth processes the bacteria are attached to a fixed surface (filter media) contained within a filter structure (Metcalf and Eddy, 1991). Attached-growth processes offer several advantages compared with suspended-growth processes:

1) One of the key advantages, though the mechanism is not fully understood, is the positive influence of solid surface attachment on bacterial activity (Hattori and Edeline, 1981; Doran and Bailey, 1986; Klein and Ziehr, 1990). This effect has been observed in different types of bacteria including autotrophic cultures of nitrifying bacteria (Bazin *et al.*, 1982; Audic *et al.*, 1984; Keen and Prosser, 1988; Bock *et al.*, 1991).

2) Fixed cultures are less strongly affected than suspended cultures by changes in environmental conditions such as temperature, pH, nutrient concentrations, metabolic products and toxic substances. This effect has been observed in different types of bacteria including autotrophic cultures of nitrifying bacteria (Duddles *et al.*, 1974; Olem and Unz, 1980). Consequently, attached-growth biological processes have a better capability to handle shock loading compared with suspended-growth processes (Hagopian and Riley, 1998).

3) Attached-growth biological processes can be easily used in small-scale treatment (Fitch *et al.*, 1998).

A fixed biofilm attached to a solid surface of the support media is mainly composed of bacterial cells, exopolymers and absorbed organic matter, creating a complex and non-homogeneous spatial structure (Lazarova and Manem, 1995). Biofilm thickness and structure depends on hydrodynamic and operating conditions and determines the transfer of nutrients into the biofilm (Characklis, 1981; Siegrist and Gujer, 1985; de Beer *et al.*, 1994). A diffusion process controls this transfer of nutrients, where a concentration gradient and a particle charge counter-flow has to exist between the

bulk liquid and biofilm (LaMotta, 1976; Haug and McCarty, 1972). Characklis *et al.* (1982) reviewed the diffusion coefficients of a considerable number of nutrients, showing that the values vary by 40-150% compared with those measured in water. Thus, as highlighted by Rasmussen and Lewandowski (1998); the kinetics of biofilm reactions is influenced by mass transport process. Harremoes (1978) suggested that below a certain concentration of one of the substrates (ammonia, nitrite and oxygen) the removal rate of ammonia and nitrite is diffusion rate limited and is proportional to the square root of the concentration of the limiting substrate (1/2-order kinetics). Above these substrate concentrations the removal rate depends only on the amount of nitrifying bacteria present and is described as reaction rate limited (0-order kinetics).

However, biofilm activity is not directly proportional to the quantity of fixed biomass because two different biofilm zones exist, including an outer 'active thickness' and an inner 'inactive thickness' (Kornegay and Andrews, 1969; LaMotta, 1976). In the latter, closer to the support media interface, the diffusion of nutrients becomes a limiting factor. Horn (1994) suggested that nitrifying populations on the surface are under ammonia-limited conditions, while those deep within the biofilm are maintained by endogenous respiration under oxygen-limited conditions, in which an anaerobic environment is established near the surface of the media.

Autotrophic organisms typically grow at significantly lower rates than heterotrophs (Metcalf and Eddy, 1991; Figueroa and Silverstein, 1992; Timmons and Losordo, 1994). Thus, in the circumstances where carbon sources are available, nitrifying biofilms are heavily overgrown by heterotrophs, which usually dominate a biofilm or bio-particle surface, and utilize oxygen before it reaches the underlying nitrifiers,

creating pH and oxygen concentration gradient problems (Siegrist and Gujer, 1987; Manem and Rittman, 1992; Zhang *et al.*, 1995; Holben *et al.*, 1998). Various authors (Antonie, 1976; Grady and Lim, 1980; Pano and Middlebrooks, 1983; Heinsbroek and Kamstra, 1990; Verhagen and Laanbroek, 1991) found that ammonia removal in biofilters was affected by organic loading. When the BOD:TKN ratio is elevated, heterotrophic bacteria enjoy an increased competitive advantage, and this can inhibit nitrification (Siegrist and Gujer, 1987; Bovendeur *et al.*, 1990; Manem and Rittman, 1992; Zhang *et al.*, 1995). Finally, Hovanec and DeLong (1996) observed that, even in RAS biofilters supplied with high percentages of N as ammonia, nitrifiers represent only 20% of the total bacterial population.

1.3.3 Nitrification in filters

As the risk of shock loading may occur in fish culture, and because of operational requirements, the inherent stability of attached-growth biological processes would appear to be the best choice for RASs (Hagopian and Riley, 1998; Bovendeur *et al.*, 1987). There are however many types of biological filters used in aquaculture, which come in a variety of physical configurations (Timmons and Losordo, 1994; Wheaton, 2002):

1) Submerged biofilters where the water flows from the top to the bottom (down flow) or from the bottom upward (up flow). In these filters the oxygen is delivered to bacteria from the water and possibly from aeration or oxygenation of the filter bed.

2) Trickling filters where the water flows from the top to the bottom. The filter media is kept wet by a thin film of water but not submerged and therefore air can circulate bringing extra oxygen to the bacteria. Moreover, these filters have an important additional function in degassing and re-oxygenating the water (Kamstra *et al.*, 1998).

3) Rotating biological contactors (RBC): 'Biodrums' where the water flows perpendicularly to a rotating perforated cylindrical container filled with the media and partially submerged in water. 'Biodisk' filters are similar to 'Biodrum'systems, though with disks spaced slightly apart along the axis. In both types of filters, the filter media is alternatively submerged in the water and exposed to the air providing a good oxygenation to bacteria.

4) Fluidised beds where high upward water flow rates are able to fluidise the media kept in the column. However, the flow rate is insufficient to wash the media out of the filter. Although the oxygen is supplied only from the water, the high flow rate provides high level of oxygenation to the bacteria.

5) Bead filters where the water flows from the top to the bottom (down flow) or more often from the bottom upward (up flow). The main characteristic of these types of filters is the filter media used, which consist of plastic beads having a specific gravity close to that of water, usually slightly less, so they float. The flow is periodically stopped causing the beads to fall and became dispersed. Then the filter is drained and when the water flow is started again the initial filter effluent, containing high solids concentrations, is discharged. Alternatively, mechanical stirring is used to disturb the beads.

The degradation of ammonia, nitrite and BOD is correlated to the activity of individual bacteria, and to the number of bacteria, attached to the fixed surface on the filter media (Horowitz and Horowitz, 2000). As a result, various types of media with different specific surface areas have been developed to hold the biological community within the filter structure. The specific surface area (SSA) is defined as the surface area of the media per unit of volume. Because the cost of building a bio-filter can be proportional to its size, the SSA of a media is an important factor in its design (Timmons and Losordo, 1994).

Generally, a small media size has a higher specific surface area and a lower void ratio compared with a large media size of the same type. Void ratio is defined as the volume of air present in a filter after it is filled with media divided by the total volume of the empty filter (Timmons and Losordo, 1994). Low void ratios increase solids accumulation and encourage the growth of heterotrophic bacteria, reducing substrate (ammonia, nitrite and particularly oxygen) availability for the desired nitrifying autotrophic bacteria (Golz *et al.*, 1999). Moreover, as it has smaller pore spaces, the smaller the diameter of the filter media, the faster the filter bed will block, requiring more frequent backwashing and, as a consequence, a higher system water consumption. Figure 1 illustrates clogging and channeling of water through a biofilter. These processes may affect the operation capacity of biofilter, mainly by reducing the active contact area and the contact time of wastewater with active biofilm (Rosenthal, 1999).

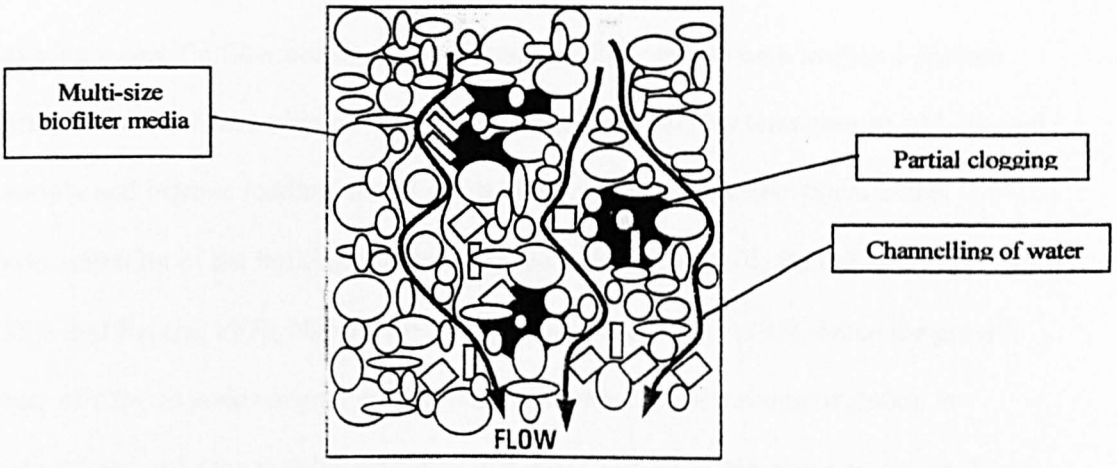

Figure 1: Processes of clogging and channeling of water through a biofilter (Rosenthal, 1999).

There are many types of filter media used in aquaculture, including sand, gravel and rocks, random plastic shapes and modular plastic units. Muir (1982) reviewed specific surface area, void ratios and weight per unit volume for different types of filter media used in aquaculture. Muir (1982) also developed an index of 'Blockability', which is defined as Specific surface area/ % Void: hence theoretical film thickness on a single surface before 100% blockage. Generally, submerged filters require a heavier filter structure compared with trickling filters that are lighter and possibly simpler (e.g. cages of simple media). As a result, submerged filters use many types of media from sand and rocks to plastic shapes, while trickling filters usually use plastic media, primarily due to their low weight per unit volume. Biodrum filters, because of their structure, use plastic shapes that allow easy handling of the filter media inside the rotating contactors and light weight to reduce power demand in moving the filter. Fluidised beds usually use sand as a filter media, which has a very high specific surface area and heavy weight to allow fluidization and to prevent wash out of the media. Because of the high void ratio (ratio between the empty volume and the total volume) a good index of 'Blockability' is expected for fluidised beds.

Giving a specific filter configuration, including filter media with available surface area and stable / favorable environmental conditions such as temperature, pH, oxygen supply and organic loading, nitrification removal rates are proportional to the concentration of the limiting substrates (Liao and Mayo, 1974; Heinsbroek, 1990; van Rijn and Rivera, 1990; Nijhof, 1995; Nijhof and Klapwijk, 1995). Since the growth rate of *Nitrosomonas* is greater than that of *Nitrobacter*, ammonia oxidation is considered to be the rate-limiting step in the conversion of ammonia to nitrate (Water Pollution Control Federation, 1983). However, several researchers (Forster, 1974; Kaiser and Wheaton, 1983; Nijhof, 1994a; Nijhof and Klapwijk, 1995) have shown that the hydraulic loading of the bio-filter strongly affects the ammonia removal rate.

The hydraulic loading is defined as the amount of water delivered to the filter per unit area of filter top surface per unit time ($m^3/m^2/day$ – or m/day) (Timmons and Losordo, 1994). These authors measured a higher ammonia mass removal rates ($g/m^3/day$) at low ammonia concentrations and higher flow rate (shorter retention time). Consequently, these results also suggest that retention time was the primary factor affecting removal (Muir, 1982). Liao and Mayo (1974) found a direct relation between ammonia removal and media retention time at various ammonia loading rates including those for aquaculture systems, in which very low ammonia concentrations are commonly found compared with those in conventional wastewater systems.

Nitrifying systems oxidise ammonia via nitrite to nitrate, but where the oxidation of ammonia is incomplete, nitrite will accumulate in RASs. Thus, high removal rates of ammonia by the filter do in themselves predict satisfactory performance. This

phenomenon is often attributed to environmental factors including pH (Alleman, 1985; Fenton and Mills, 1980), substrate and product inhibition (Anthonisen *et al.*, 1976; Focht and Verstraete, 1977), and light intensity (Olson, 1981; Diab and Shilo, 1988). In particular, nitrite accumulation often takes place under condition of low oxygen concentrations, where oxygen limits nitrite oxidizers at higher concentrations than it does for ammonia oxidizers (van Rijn and Rivera, 1990). However, in various field studies (van Rijn and Rivera, 1990; Nijhof and Klapwijk, 1995; Kamstra *et al.*, 1998) increased levels of nitrite in fish culture occurred also when these factors were within the range that should not have caused nitrite accumulation. These researchers suggested that such events are probably caused by a relatively low nitrite removal rate compared to that for ammonia in the biofilm.

Nitrite accumulation in RASs could be also explained by substrate limited diffusion process (Nijhof and Klapwijk, 1995). Ammonia from the bulk fluid penetrates the biofilm producing nitrite within the biofilm itself and creating a nitrite concentration gradient between bulk fluid and the upper biofilm layer, which causes a partial outward diffusion of nitrite (Bovendeur, 1989). An equilibrium situation can be eventually achieved, resulting in no net outward diffusion nitrite and thus complete oxidation of ammonia to nitrate, when the bulk fluid nitrite concentration is equal to the nitrite concentration of the upper biofilm layer. However, it seems generally difficult to predict and control nitrite level in RASs because the nitrite removal capacity in nitrifying systems is variable and sensitive to environmental disturbance (Kamstra *et al.*, 1998).

From what it has been illustrated above it would seem that there is a great potential in exploring new materials for the usage as alternative reactive biofilter media, able to improve performance of biological treatment in RASs. These types of reactive media could be able to interact differently with bacterial substrates, for example by adsorption and/or reaction processes, enhancing biological performance. In this context, this study aims to investigate the application of manganese dioxide ore as an alternative supporting media in biological filters.

1.4 The aim of the project

In order to improve biological filter efficiency and reliability, numerous studies have focused on comparing different biofilter designs and different types of filter media (Liao and Mayo, 1974; Kruner and Rosenthal, 1983; Rogers and Klemetson, 1985; Bovendeur *et al.*, 1987; van Rijn and Rivera, 1990; Westerman *et al.*, 1993; Drennan *et al.*, 1993; Tsukuda *et al.*, 1997). The research objectives of this project examined the capacity of manganese dioxide, as a supporting media in bio-filters, to remove metabolites (especially nitrite) reliably and more efficiently compared with existing technology, as well as improving safety of aquaculture systems.

Manganese dioxide possesses catalytic oxidation properties, and is the most generally favored media for iron and manganese removal in water treatment for human consumption. Consequently its applications in this specific field are well characterized. However, no published studies have been found that specially examine its usage in the elimination of nitrogen-containing metabolites, and a search of the

literature confirmed that manganese dioxide has not previously been used in closed system aquaculture.

The investigation on manganese dioxide as supporting media in bio-filters within RASs, was initially informed by the following evidence:

1) Manganese forms compounds with oxidation states ranging from +II to +VII in aqueous conditions, but under conditions of dilute acid only Mn_2^+, MnO_2 and MnO_4^- (i.e. the II, IV and VII formal oxidation states) are stable. In general terms, Mn (VII) compounds are strongly oxidising, Mn (IV) compounds are oxidising and Mn (II) is stable with regard to reduction. Permanganate (Mn VII) is well known as an oxidising agent for the conversion of nitrite (NO_2^-) to nitrate (NO_3^-) and it might be expected from thermodynamic arguments that manganese dioxide (MnO_2) would also oxidise NO_2^-.

2) Though the methods used were not described in literature, typical specific surface areas developed by plastic media are 200 - 300 m^2/m^3 (Muir, 1982), while sand media, depending on the grade size, typically have a specific surface area of around 2000 - 3000 m^2/m^3 (Westerman, *et al.*, 1993). Manganese dioxide exhibits a high specific surface area (Morgan and Stumm, 1965), when measured with classical N_2 adsorption methods (Brunauer *et al.*, 1938), in the order of 100 – 200 $m^2\ g^{-1}$, which is the equivalent of approximately 2 x 10^8 - 4 x 10^8 m^2/m^3 (Murray, 1973; Balistrieri and Murray, 1982). This could lend to a significantly increased efficiency over biological

filters based on surface areas quite apart from performance, which is chemical in origin.

3) Conventional bio-filters hold a diverse biological community generally composed of nitrifying bacteria and heterotrophic bacteria, which are in constant competition for oxygen uptake (Timmons and Losordo, 1994; Golz *et al.*, 1999). With manganese dioxide as a supporting media in bio-filter, it is likely that a different biological community will develop. It is known that because of iron and manganese levels, manganese dioxide filter media could enhance the development of a different bacteria community of iron bacteria within the filter bed (Buzio and Alberti, 1995). These will compete with nitrifying and heterotrophic bacteria for surface space and also for oxygen uptake, depending on the species of iron bacteria colonizing the bio-filter. As a result, the presence of iron bacteria could also modify the competitive relation between nitrifying bacteria and heterotrophic bacteria. The variation in performance of biological filters adopting manganese dioxide may also relate to biological changes occurring inside the bio-filter bed.

However, the primary concern about the application of manganese dioxide in an aquaculture context is represented by the high toxicity of manganese to fish. Exley and Phillips (1988) in their review on the chemistry, toxicity and the ameliorative properties of the most common trace metals, reports acute effects of manganese at > 770 $\mu g\ L^{-1}$ and sub lethal gill damage at 100 – 500 $\mu g\ L^{-1}$, and that calcium is considered the main antagonist to reduce manganese toxicity.

The aim of this project was to investigate the usage of manganese dioxide as a biological media to remove ammonia and nitrite in RASs, and to define the relative significance of physical, chemical and biological factors, singularly and/or interrelated, and the rate of manganese solubility in the performance. Manganese dioxide filter specifications were also developed and related to financial performance parameters including total cost per unit of metabolites removed, feed consumption and stock produced.

- Following this introduction, the next chapter (2) describes the chemical properties of manganese dioxide, its application to water treatment, and the manganese oxidation and reduction occurring in the natural environment.

- Chapter 3 illustrates the experimental trials carried out at the Institute of Aquaculture in the University of Stirling. The aim was to evaluate physical and chemical properties of manganese dioxide in the absence of biological activity. Total surface area developed by manganese dioxide ore was measured and compared, including surface morphology observations, with different types of commercial bio-filter media. Secondly, static batch trial and a mini-recirculation systems including manganese dioxide submerged filter were used to test manganese solubility and to measure adsorption and reaction of ammonia, nitrite and nitrate with manganese dioxide. During the batch trial a silicate sand medium was chosen as a control to investigate chemical properties because it is commonly used in biological filters.

- The next chapter (4) illustrates the experimental field trials carried out at a full scale commercial recirculating water salmon hatchery*. Two full-scale pilot filters were installed in order to compare metabolite removal performance of silicate sand and manganese dioxide ore in condition of biological activity commonly found in bio-filters.

- Chapter 5 describes a technical and financial analysis based on metabolite mass balance and capital and operational cost in a commercial salmon hatchery recycle system. Based on performance data available at the commercial farm, the study simulated the substitution of the existing trickling filter with bio-filters that use manganese dioxide technology.

- The final chapter presents the discussion and conclusions.

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* *Alexander Sandison & Sons Ltd was founded in 1927 and still maintains the head office in Unst - Shetland. One of the main businesses is now the operation of Quoys salmon hatchery, which contributes about 40% of Shetland's smolt production.*

Chapter 2
A conceptual background on manganese dioxide

2.1 Chemical background

2.1.1 Properties of hydrous manganese dioxide

Manganese occurs in a broad range of minerals that are widely distributed in the earths crust. It constitutes about 0.1% of the earth's crust and is the twelfth most abundant element. Since 1960, the principal sources of commercial grades of manganese ore have been Australia, Brazil, Gabon, India and Republic of South Africa. Commercial samples exist as brown granular powder from natural crushed ore with a manganese content of 70 - 75% as MnO_2[1], characterized as pyrolusite (β-MnO_2) with high degree of crystallinity (Di Ruggiero, 1989; Rusin *et al.*, 1991). In contrast, this oxide seems to have a poor degree of crystallinity in its δ-MnO_2 form, while the γ-MnO_2 form is more crystalline (Morgan and Stumm, 1964; Gabano *et al.*, 1965).

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[1] *MnO_2 as a formula reflects the compositions of these manganese minerals and commercial compounds only to a first approximation, in that the stoichiometric ratio, n ([O]/[Mn]) overall = two, and manganese is present primarily as Mn (IV). However, in reality this stoichiometry is not attained throughout these compounds, with n varying from >1.500 to <2.000. Consequently, MnO_2 is a non-stoichiometric compound, which may be considered as MnO2-MnOOH solid solution, where the manganese is present in oxidation states of +4 and +3 (Gramm-Osipov et al., 1991).*
Furthermore, the disproportionation reaction (equation [1]) means that in solution non-stoichiometric manganese dioxide exists in equilibrium with an electrolyte at given Mn^{2+} and H^+ concentrations:

$$2MnOOH + 2H^+ \Leftrightarrow MnO_2 + Mn^{2+} + H_2O \qquad [1]$$

Experimental evidence exists for manganese dioxide being significantly more soluble than theoretical thermodynamic calculations would suggest (Savenko, 1985; Swain *et al.*, 1975; Gramm-Osipov *et al.*, 1991). Figure 2 shows a p*E*-pH diagram for manganese constructed using thermodynamic data available in the literature.

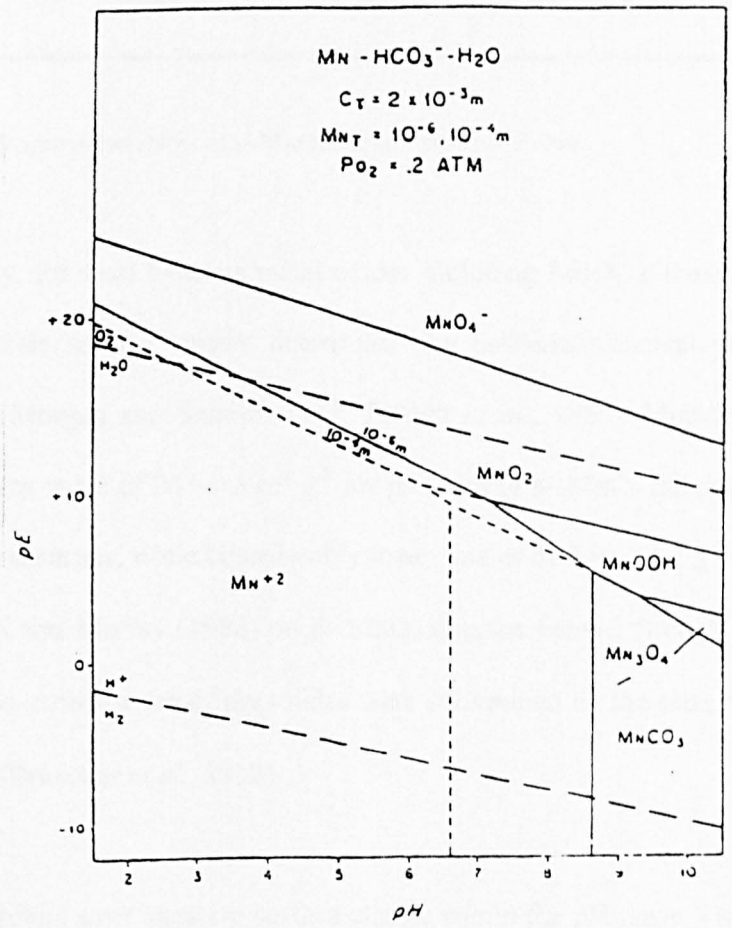


Figure 2: pE-pH diagram for manganese.

From the relative positions of the MnO_2 -Mn^{2+} and O_2 -H_2O lines it would appear that δ-MnO_2 becomes unstable with respect to the oxidation of water at pH values below 3.5. However, Gramm-Osipov *et al.* (1991) reports that γ-MnO_2 suspension releases Mn ion at pH = 5.99 (Table1).

Treatment time (day)	Initial (*n*) Stoichiometric ratio [O]/[Mn]	Final (*n*) Stoichiometric ratio [O]/[Mn]	Initial pH	Final pH	[H+] 10E6 M	[Mn2+] 10E6 M
3	1.829	1.830	5.99	6.35	- 0.56	4.91
6	1.762	1.792	5.95	6.68	- 0.91	5.1

Table 1: Manganese solubility of γ- MnO_2 suspension in pure water.

Generally, for most hydrous metal oxides including MnO_2, the extent and character of the oxide surface largely determines the colloidal-chemical properties of this material (Morgan and Stumm, 1964; Posselt *et al.*, 1968). Murray (1973) found a surface area value of 263 ± 5 $m^2 g^{-1}$ for particles of δ- MnO_2 ranging from 0.2 to 1.0 μm mean diameter, while considerably lower values of 74 ± 1 $m^2 g^{-1}$ were reported by Balistrieri and Murray (1982) on δ- MnO_2 samples passed through a fine (400 μm) sieve. The surface areas of the oxides were determined by the classical N_2 adsorption methods (Brunauer *et al.*, 1938).

MnO_2 exhibits a net negative surface charge within the pH range 5 to 11, of principal interest for natural waters and for water treatment operations. Figure 2 represents a schematic arrangement of surface atoms for MnO_2 proposed by Posselt *et al.* (1968) and similar to that proposed by Gabano *et al.* (1965). As suggested by the configuration shown in Figure 3, which assumes a relatively well-ordered crystal, surface-bound hydrogen and hydroxide ions may exchange at the surface of the MnO_2 in response to changes in the relative activities of their ions in solution phase. Thus, at least in the absence of other ionic species, H^+ and OH^- function as potential-

determining ions, and the surface charge of the MnO_2 is largely determined by the pH of the solution. The surface charge becomes more negative as pH increases as a result of the increased ratio of bound OH^- to bound H^+.

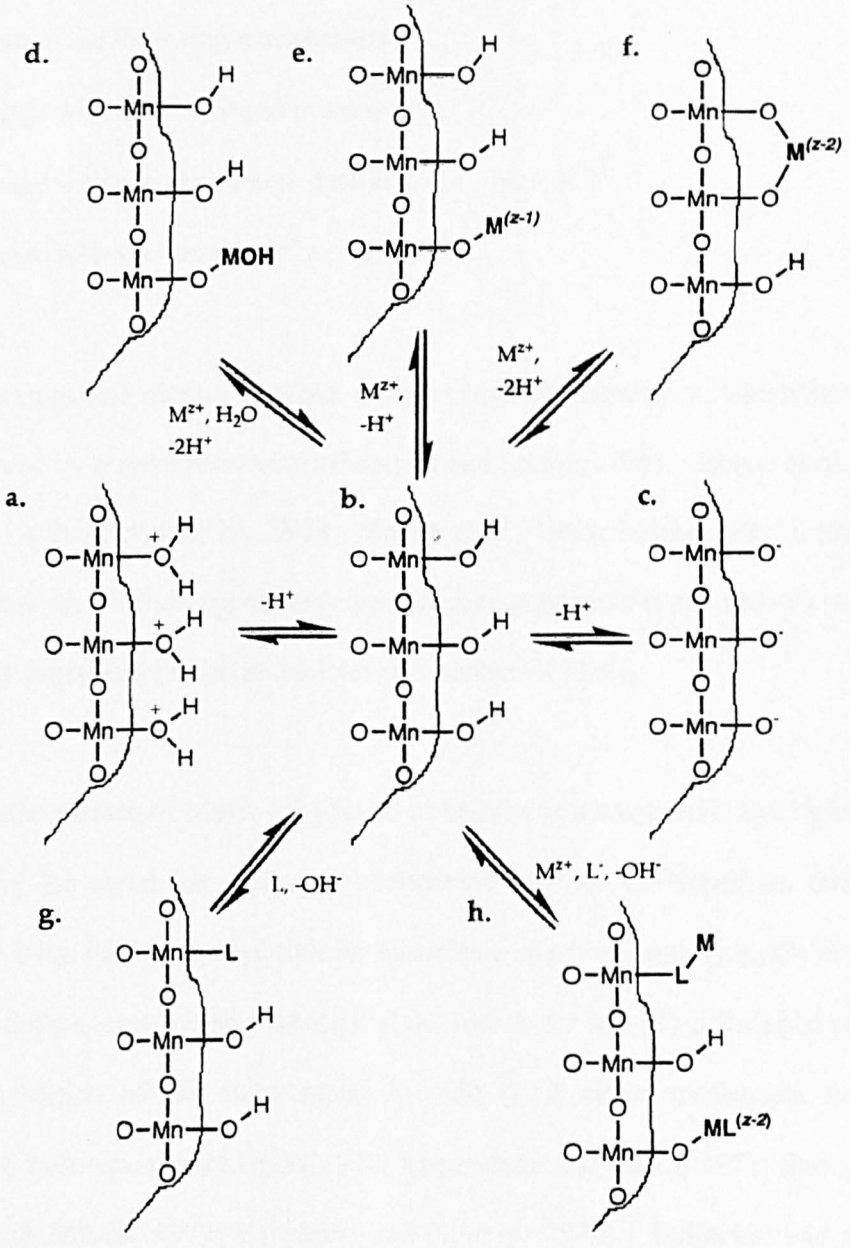


Figure 3: The solution behavior of MnO_2 showing protonation/deprotonation steps (6a, b and c); Metal biding (6d, e and f); Ligand exchange (6g) and two types of ternary complex formation.

2.1.2 Sorptive characteristics of hydrous manganese dioxide

Interactions with cationic species:

The adsorption of metals ions is believed to occur (Balistrieri and Murray, 1982) by one or more of the following mechanisms:

- Exchange with surface bound protons
- Exchange with adsorbed electrolyte ions (*i.e.*, Na+, K^+)
- Exchange with structural Mn^{2+} or Mn^{3+}

Hydrous manganese dioxide exhibits ion exchange characteristics, which have been demonstrated by several researchers (Morgan and Stumm, 1965; Gabano *et al.*, 1965; Kozawa, 1959; Murray *et al.*, 1968; Posselt *et al.*, 1968; Murray, 1975). From the results obtained, it would appear that these exchange properties are mainly associated with acidic functional groups at the hydrated surface of MnO_2.

The hydrated surface of MnO_2 is believed to behave as a weak acid, and logically an explanation for metal ion exchange phenomena may be developed on this basis. Moreover it has been suggested that the adsorption of trace metals (e.g., Co or Zn) by MnO_2 partially occurs by the exchange of the metals for Mn (II) in the solid phase or by the oxidation of the trace metal by Mn (IV); either mechanism releasing manganese to solution (McKenzie, 1970; Loganathan and Burau, 1973; Burns, 1976; Murray and Dillard, 1979; Balistrieri and Murray, 1982). Balistrieri and Murray (1982) failed in their experimental measurements to show the release of Mn to solution during the adsorption of Mg and Ca^{2+}. This was explained by the fact that

adsorption of Mg^{2+} and Ca^{2+} occurs by exchange with Na^{+} and K^{+} ions as well as protons, where equivalent charge is adsorbed and release on the δ- MnO_2 surface.

In major ion sea water at pH=8, 84.4% of the surface sites on δ- MnO_2 are complexed by H^{+}, 8.4% by Mg^{2+}, 4.6% by Ca^{2+}, 1.6% by Na^{+}, and 0.6% by K^{+} (Balistrieri, Murray, 1982). Murray (1975) has suggested that the affinity of the metals for the surface of synthetic sample of hydrous manganese dioxide followed the order:

$$Mg < Ca < Sr < Ba < Ni < Zn < Mn < Co$$

The relative bond strength is related to the specific adsorption potential, which is determined from the amount of metal that is adsorbed by the surface, in absence of any electrostatic attraction, at the pH of zero point of charge (Murray, 1975). It is widely accepted that the order of exchange affinity is a function of ionic size. Within a given group of elements the crystalline ionic radius is proportional to the exchange affinity (Posselt *et al.*, 1968). The greater sorptive capacity of hydrous MnO_2 for the Mn^{2+} ion compared with other divalent ions could be explained by the specific equilibrium established between the surface of the MnO_2 and the Mn^{2+} ions in solution demonstrated by Gramm-Osipov *et al.* (1991).

Ionic strength and pH have been investigated as major system variables on sorptive characteristic of hydrous MnO_2. The effect of pH on the sorption of metal ions by hydrous MnO_2 has been studied (Gabano *et al.*, 1965; Morgan and Stumm, 1965; Posselt *et al.*, 1968) and, in general, the results for all of the metal ions studied follow a pattern much like that established by Morgan and Stumm (1964) for Mn^{2+} (Figure 4). Posselt *et al.* (1968) investigated the effects of ionic strength on the sorption of metal ion by hydrous MnO_2. The authors conducted equilibrium measurements on the

sorption of Ca^{2+}, which showed that sorption capacity decreases with increasing ionic strength. Nevertheless, the decreased sorption capacity could be simply the effect of an increased concentration of Na^+ and ClO_4^-, which were used to adjust the ionic strength of the solution.

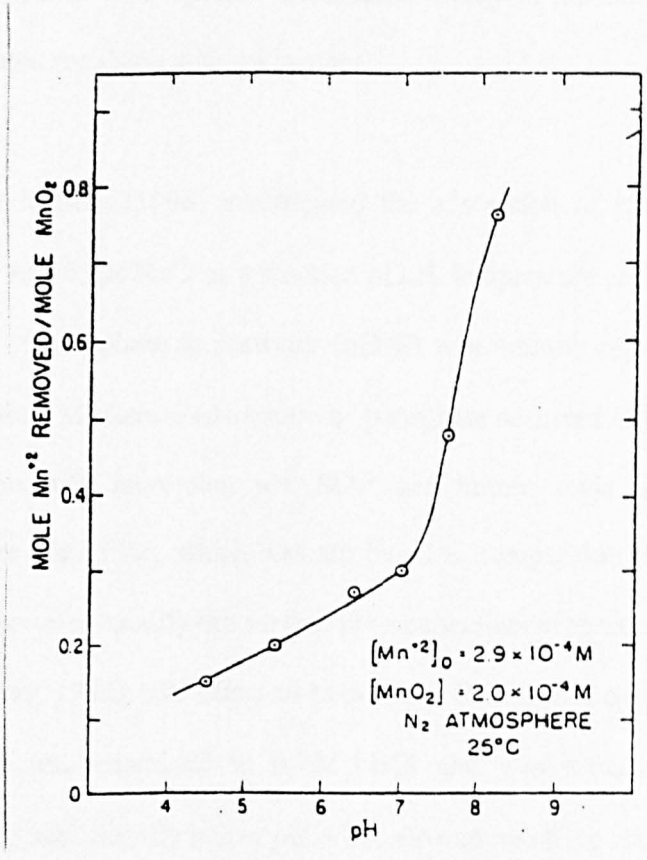


Figure 4: pH-dependent sorption of manganese (II) on manganese dioxide.

Interaction with anionic species:

MnO_2 has negative surface charges near neutral pH and so is thought to have a limited capacity to adsorb anions (Yao and Millero, 1996). Nevertheless the surface charge of MnO_2 may be reversed by exchange of the monovalent surface protons for divalent cations from solution (Murray, 1975). However, little published work has been reported on the adsorption of anions on MnO_2. Balistrieri and Murray (1982)

investigated the surface chemistry of δ- MnO_2 in major ion seawater. In this study it was reported that chloride and sulphate do not adsorb on δ- MnO_2 in the pH range of natural water. These workers suggest that since the zero point of charge of δ- MnO_2 is ≈1.5, the negatively charged surface effectively prevents the adsorption of these anions because their specific adsorption energy is not sufficient to overcome the electrostatic repulsion with the surface.

Yao and Millero (1996) investigated the adsorption of phosphate on δ- MnO_2 in seawater and 0.7*M* NaCl as a function of pH, temperature and salinity. The adsorption reaction of phosphate in seawater (pH=8) was initially rapid with 60% completion after 5 min. Maximum adsorption of phosphate occurred at low pH, with decreasing adsorption with increasing pH. SO_4^{2-} and humic acids were found to suppress phosphate adsorption, which was attributed to competition for surface sites. Humic acid is known to modify the surface physiochemical properties of particles (Balistrieri and Murray, 1982). The effect of humic acid (10 mg L^{-1}) on phosphate adsorption on δ- MnO_2 was examined in 0.7*M* NaCl and was found to suppress phosphate adsorption significantly below pH 5 but showed no effect above pH 6. Kawashima *et al.* (1986) stated that in the presence of alkaline earth cations and transition metal ions, hydrous manganese dioxide strongly adsorbs phosphate between pH 6.0 and 9.0. The addition of Ca^{2+} and Mg^{2+} in 0.7*M* NaCl was found to significantly enhance the adsorption of phosphate on δ- MnO_2 at pH> 4. This increased adsorption might be due to the change in both the surface of δ- MnO_2 and the solution speciation of phosphate in the presence of Ca^{2+} and Mg^{2+} (Yao and Millero, 1996).

Interaction with organic species:

As mentioned by Tipping and Heaton (1983), several studies in the early 80's have demonstrated that the adsorption of soluble organic matter plays an important part in the surface chemistry of oxides in natural waters. The adsorption of humic substances affects the colloid stability, particle growth, crystallization and dissolution of, and interactions with other chemical species by MnO_2. Tipping and Heaton (1983) investigated the adsorption of natural organic matter (humic substances) by two synthetic oxides of manganese. The addition of bivalent cations (*i.e.*, Ca^{2+} in $0.01M$ NaCl) was found to enhance the adsorption of humic substances while increasing pH seemed to decrease the adsorption properties of the manganese oxides. The humic adsorption on oxides involves the uptake of proton (or expulsion of hydroxyl ions) for the following reasons:

1) To accomplish ligand exchange, where humic anionic groups exchange with H_2O or OH^- and / or by surface complexation between oxide OH_2^+ groups and humic anionic groups (Davis, 1980, 1982; Tipping, 1981a,b).

2) To neutralize the negative charges of humic anionic groups not involved in the adsorption interaction, in order to overcome electrostatic repulsion when humic substances concentrate at the interface (Hunter, 1980; Tipping, 1981a; Tipping and Cooke, 1982).

2.2 Redox chemistry of nitrogen and manganese

The redox chemistry of nitrogen is complex, reflecting the wide range of formal redox states available to the element from -III (NH_3) to +V (NO_3^-). These redox potentials are also pH dependent, for example, in acid conditions (pH=0)

$$2NO_3^- + 3H^+ + 2e^- \leftrightarrow HNO_2 + H_2O \qquad E^\circ = +0.94V$$

while in basic conditions (pH=14)

$$NO_3^- + H_2O + 2e^- \leftrightarrow NO_2^- + OH^- \qquad E^\circ = +0.01V$$

The oxidation of NH_3 (NH_4^+) to NO_3^- proceeds via a number of potential intermediates such as hydroxylamine, NH_2OH, and nitrite NO_2^-. However, the redox chemistry is further complicated by the existence of disproportionation (or even conproportionation) reactions such as (in acid conditions)

$$4NH_3OH^+ \leftrightarrow N_2O + 2NH_4^+ + 3H_2O + 2H^+ \quad \text{(slow)}$$

or in alkali conditions

$$3NH_2OH + \leftrightarrow N_2 + NH_3 + 3H_2O \quad \text{(fast)}$$

or in the case of nitrous acid at room temperature in acid solution, the following reaction is observed

$$3HNO_2 + \leftrightarrow H_3O^+ + NO_3^- + 2NO$$

However, it must also be understood that these are thermodynamic arguments and observed under standard conditions. Substantial kinetic barriers may exist to some or all of these processes even under standard conditions and under the proposed conditions of study, in the presence of manganese dioxide, heterogeneous catalytic processes may substantially alter the kinetics of these processes.

Manganese is the fifth most abundant metal on the surface of the earth and the second most abundant transition metal after iron. Manganese forms compounds with oxidation states ranging from +II to +VII in aqueous conditions, but under conditions of dilute acid only Mn_2^+, MnO_2 and MnO_4^- (i.e. the II, IV and VII formal oxidation states) are stable. Disproportionation reactions of other Mn oxidation states occur in acid conditions. The relevant redox processes for MnO_2 in acid and alkali solution are respectively

$$MnO_2 \leftrightarrow Mn^{2+} \qquad E^\circ = +1.23\ V$$

$$MnO_2 \leftrightarrow Mn(OH)_2 \qquad E^\circ = -0.05\ V$$

In nature manganese is commonly found with an oxidation state of II, III, or IV. In natural waters the soluble form occurs primarily as the biologically available free cation (Mn^{2+}), while Mn (III) and Mn (IV) form a variety of insoluble oxides and

oxyhydroxides (Balistrieri and Murray, 1982; Murray, 1974; Murray and Dillard, 1979). At the pH and E_h values found at the oxic conditions typical of the Earth's surface, the manganese redox state equilibrium is shifted to Mn (III) and Mn (IV). However, soluble manganese can be quite stable in natural waters. This is because the activation energy of Mn (II) oxidation is large and as a result oxidation proceeds very slowly (Stumm and Morgan, 1981). In spite of this, the oxidation of Mn (II) is pH dependent and the reaction becomes rapid at pH values above 9.0. To further complicate the manganese equilibrium, Mn (II) oxidation is autocatalytic; once oxidation begins, the freshly formed MnO_x adsorbs Mn (II) and greatly increases the rate of reaction.

In general terms, Mn (VII) compounds are strongly oxidising, Mn (IV) compounds are oxidising and Mn (II) is stable with regard to reduction. Permanganate (Mn VII) is well known as an oxidising agent for the conversion of NO_2^- to NO_3^- and it might be expected from thermodynamic arguments that MnO_2 would also oxidise NO_2^-.

2.3 The application of manganese dioxide to water treatment

MnO_2 possesses catalytic oxidation properties, representing one of the favored media for iron and manganese removal in water treatment for human consumption and consequently its application in this field are well characterized (Cox, 1964). However, no published studies have been found that specially examine its usage in the elimination of nitrogen-containing metabolites.

Cox (1964) suggests various treatment processes of iron and manganese removals as a function of different characteristics of water. Contact filtration with MnO_2, pyrolusite, is considered suitable only if iron and manganese are bound to organic matter but with no excessive carbon dioxide or organic-acid content. In the case of coloured turbid surface water containing iron and manganese in combination with organic matter and organic acid, pH control and chemical treatments with ferric chloride, or chlorinated copperas[2], or lime followed by sand filtration are listed among the treatment process suggested.

More recently Universal mineral supplied Ltd. (personal communication) suggests that for the removal of iron and manganese from potable water, using MnO_2 combined with continuous pre-chlorination to break point, the presence of free ammonia results in the equilibrium formation of hypochlorous acid, HOCl, throughout the filter bed:

$$3MnO_2 + 3HOCl + 5OH^- \rightarrow 2MnO_4^- + 3Cl^- + 4H_2O$$

HOCl is a strong oxidizing agent, and is the most common disinfecting agent used in water treatment. HOCl is capable of oxidizing MnO_2 to permanganate. The chlorine produced by this process is in turn reduced to chlorine ion (Cl^-). The permanganate created around each grain of MnO_2 media rapidly oxidizes soluble Mn (II) to form MnO_2. The permanganate in turn is reduced back to MnO_2 and both products adsorb

[2] *Iron sulphate associated with chlorine molecules:* $FeSO_4.7H_2O + 1/2Cl_2$

on the surface media. The greater the surface area of MnO_2 in the filter bed, the more efficient the treatment process. The standard catalytic filter consists of 80% standard filter sand 16/30 grades, and 20% MnO_2 18/44 grades. Moreover UMS Ltd claims that the grain of MnO_2 would be able to 'condition' or 'sensitize' the surrounding sand grains and these then also perform an oxidation function. UMS Ltd. explains the phenomenon by suggesting that the sand grains would become coated with manganese oxides precipitated from the water being treated.

Biological manganese oxidation is also exploited in water treatment for Fe (II) and Mn (II) removal. Various procedures are used, including slow filtration, floating filtration and rapid filtration. In particular, rapid filtration occurs in biological reactors (Mouchet, 1992; Buzio and Alberti, 1995). Mn-containing water is aerated and injected through rapid sand filters where aquatic bacteria (e.g. *Leptothrix*) develop and precipitate manganese. *Leptothrix* were also found to be dominant bacteria on sand filters (Xambeau, 1990) and Buzio and Alberti (1995) identified the following groups of bacteria:

- Iron Bacteria
- Chemoheterotrophic bacteria belong to *Sphaerotilus-Leptothrix* groups
- Specialized chemoautotrophic bacteria (*Siderocapsa* sp. and *Leptothrix ochracea*)

The rapid filtration procedure appears to be very effective for manganese removal. Rapid filtration reactors are used in many European countries; however, physico-chemical treatments remain the rule in the United States (Gounot, 1994).

2.4 Manganese oxidation and reduction in natural environment

Manganese is a vital trace element for microorganisms (Metcalf and Eddy, 1991). This transition metal is involved in many enzymatic biochemical processes, especially in those reactions associated with oxygen (*e.g.* oxygen protection via O_2^- scavenging by Mn (II)) (Nealson *et al.*, 1988). Additionally, it is now well established that microorganisms, including bacteria, algae, yeast and fungi, can either oxidize Mn (II), or reduce, Mn (III) and Mn (IV) oxides; different microorganisms have, either directly or indirectly, a major impact on the manganese cycle in the natural environment (Gounot, 1994).

Among the wide variety of Mn oxidizing microorganisms reported by Ghiorse (1984), there are bacteria belonging to common Gram-positive or Gram-negative groups, e.g. *Arthrobacter, Micrococcus, Bacillus, Chromobacterium, Pseudomonas, Vibrio, and Oceanospirillum.* Gounot (1994) describes the Mn oxidation process as direct (enzymatic) or indirect:

- Indirect oxidation caused by the production of hydrogen peroxide, free radical or oxidant species;
- Direct oxidation in an enzymatic reaction catalysed by Mn binding and oxidizing protein has been found in crude or purified extracts.

There is evidence to suggest that autotrophic or mixotrophic bacteria could have the ability to utilize Mn (II) as a source of electrons for the immobilization of inorganic carbon into biomass (Ehrlich, 1976; Ghiorse, 1984; Nealson *et al.*, 1988; Nealson *et al.*, 1989). Moreover, evidence exists that oxidation of Mn (II) may be a protective

mechanism in opposition to Mn toxicity (Ghiorse, 1984) or alternatively against oxidant toxicity (Dubinina, 1979; Archibald and Fridovich, 1981; Ghiorse, 1984). However, although evidence has been presented, there is no unequivocal proof that can fully justify the biological significance of microbial Mn oxidation (Gounot, 1994).

Mn-reducer microorganisms would appear to be more ubiquitous in the biosphere then those capable of Mn oxidation (Gounot, 1994). Lovley (1991) described a wide variety of Mn-reducers from highly aerobic bacteria or fungi to strictly anaerobic bacteria. More than 200 strains of manganese reducers have been isolated, from various environments, consisting of different taxa including *Pseudomonas* spp., *Bacillus* spp., and many others (Nealson and Myers, 1992).

Gounot (1994), as with manganese oxidation, divided the mechanism of manganese reduction into direct (enzymatic) or indirect processes:

- The indirect reduction includes a redox model and a direct-reduction model. In the redox model, microbial metabolism lowers the pH and / or redox potential according to equilibrium thermodynamics and the Mn (II) – Mn (IV) equilibrium is increasingly shifted in favour of Mn (II). In the direct-reduction model, it is assumed that inorganic or organic compounds produced by microorganisms or present in sedimentary environments, react directly with Mn (IV) to reduce it (Lovley, 1991).
- The direct (enzymatic) reduction involves the oxidation of organic matter coupled to the reduction of Fe (III) and Mn (IV) as a direct result of the enzymatic activities of specialized microorganisms

Dissimilatory Mn (IV) reduction can be defined as the use of Mn (IV) as an external electron acceptor during metabolism (direct reduction), coupled to organic matter oxidation either in fermentation or in anaerobic respiration (Lovley, 1991). Microbial metal reduction occurs primarily in stratified, oxygen deficient environments, for example, in stagnant water columns or sediments with high inputs of organic carbon. In these types of environments, oxygen depletion can occur rapidly, leaving the microorganisms to decompose organic matter anaerobically (Froelich *et al.*, 1979).

Alternate electron acceptors utilized by microorganisms when O_2 becomes depleted are nitrate (NO_3^-), Mn (IV) compounds, Fe (III) compounds, sulphate (SO_4^{2-}) and CO_2 (Turner and Patrick, 1968). The reduction of these oxidized inorganic redox components is generally sequential, with one oxidizing agent not being reduced until all of another oxidized component had been completely reduced. For example, Patrick and Jugsujinda (1992), studying the sequence of reduction of NO_3^-, Mn (IV) compounds, Fe (III) compounds in flooded soil, found that the there was no overlap in the reduction of the NO_3^- and Mn systems and little overlap in reduction of Mn and Fe systems.

Nealson and Myers (1992), comparing the redox potential of Mn (IV) with nitrate and sulphate, argue that for both thermodynamic and kinetic reasons Mn (IV) should serve as excellent alternate electron acceptors for anaerobic respiration. Nevertheless, the energy picture is complicated because Mn (IV) forms a variety of different oxide and oxyhydroxide phases with different mineralogies, which can have substantially different redox potentials. For this reason, various researchers compared the reduction

of highly crystalline β- MnO_2 (pyrolusite) and different types of amorphous Mn oxides. Di Ruggero (1989) found that amorphous Mn oxide prepared in the laboratory was more rapidly reduced than crystalline MnO_2, while Burdige *et al.* (1992) observed identical rates of manganese reduction between pyrolusite and amorphous δ-MnO_2 (vernadite). Furthermore, *Bacillus polymyxa* strain D1 rapidly reduces pyrolusite (Rusin *et al.*, 1991).

Metal oxides, unlike nitrate or sulphate, are unusual electron acceptors because as solids they cannot diffuse through bacterial envelopes. Thus, Nealson and Myers (1992) suggest that organisms interacting with these solid substrates must have unique physical and biochemical attributes, which might include:

1. The ability to solubilize the substrate
2. The ability to attach to the substrate and directly transfer an electron to it
3. The ability to transport the substrate into the cell as a solid

Indirect manganese reduction can be explained with the redox model, where bacterial activity drops the pH and E_h value below 6.75 and 350 mV, enhancing a spontaneous reduction of manganese (Gounot, 1994). Alternatively, various inorganic and organic compounds, produced by bacterial metabolism or present in sedimentary environments, may react directly with Mn (IV) to reduce it (Lovley, 1991):

Nitrite can reduce Mn (IV) (Bartlett, 1981) either to Mn (II) or to Mn (III) as follows:

$$NO_2^- + MnO_2 + 2H^+ \leftrightarrow Mn^{2+} + NO_3^- + H_2O$$

$$NO_2^- + 2MnO_2 \leftrightarrow Mn_2O_3 + NO_3^-$$

Bartlett (1981) suggests that Mn (IV) oxidation of nitrite could explain the fact that nitrite does not generally accumulate in aerobic soils even when the activity of *Nitrobacter* sp. is low. Moreover, Mn (IV) is frequently reduced within the nitrate reduction zone of aquatic sediments, and this could potentially be the result of nitrite reduction of Mn (IV) (Lovley, 1991).

Sulphide can also chemically reduce Mn (IV) (Burdige and Nealson, 1986) to Mn (II) as follows:

$$3H^+ + MnO_2 + HS^- \leftrightarrow Mn^{2+} + S^o + 2H_2O$$

In this case MnO_2 reduction would be limited to anaerobic zones, especially in marine environments containing sulphate (Lovley, 1991).

A certain number of organic compounds can reduce Mn (IV) (Stone and Morgan, 1984; Lovley, 1991). In particular, Bermingham (2001) examined the effect of phenols on reduction of commercial manganese ore. This research attempted to mimic the behavior of humic acid, where phenol was used as a simplified model of poly-phenolic residues present in humic acid. Since humic acid possesses complex behavior that is influenced by change in pH and ionic strength, experiments were carried out in distilled, soft and hard synthetic water. Soluble manganese appeared to be proportional to the concentration of phenol in distilled and soft water, while in hard water there was not the same distinct correlation between phenol concentration and Mn solubility. From these results it seems that the ionic strength could affect solubility of MnO_2. Nevertheless, the vast majority of organic compounds usually

found in sedimentary environments do not appear to a biologically react with Mn (IV). It would appear that the extent of this mechanism is much less than that possible through microbial metabolism (Lovley, 1991; Nealson and Myers, 1992).

It is generally accepted that it is often difficult to distinguish between direct (enzymatic) and indirect Mn reduction in aquatic sediments (Lovley, 1991; Gounot, 1994). This is because manganese oxides behave as ion exchangers, having very remarkable ion exchange capacities depending on pH. Consequently, very small variations in pH and / or redox potential that might be due to bacteria metabolism could cause manganese reduction. Thus, enzymatic reduction is generally very difficult to prove because of the potential chemical interactions between redox chemistry of manganese and bacterial activity.

2.5 Potential drawbacks of MnO_2 filter systems

The primary concern about the application of MnO_2 in an aquaculture context is represented by the high toxicity of manganese to fish. Exley and Phillips (1988) report acute effects of manganese at > 770 $\mu g\ L^{-1}$ and sub lethal gill damage at 100 – 500 $\mu g\ L^{-1}$ and calcium is considered the main antagonist to reduce manganese toxicity.

An investigation of the chemical properties of hydrous MnO_2 highlight a greater solubility of solid MnO_2 than thermodynamic data available in the literature would imply. Two main mechanisms are involved (Gramm-Osipov, *et al.*, 1991; Balistrieri and Murray, 1982):

- Specific equilibria are established between the surface of the MnO_2 and Mn^{2+} ions

- Sorptive properties of hydrous MnO_2 allow exchange of metal ions with surface bound protons

The primary controlling factor in both these phenomena is pH. In general, the higher the pH, the higher will be the stability of solid MnO_2 and the sorptive capacity of metal ions on its surface.

It has also been recognized that the adsorption of trace metals (e.g., Co or Zn) by MnO_2 partially occurs by the exchange of the metals for Mn (II) in the solid or by the oxidation of the trace metal by Mn (IV); either mechanism should release manganese into solution. The net balance between Mn release and Mn removal from solid MnO_2 is determined by pH, the redox state and concentration of Mn^{n+} ions in solution.

The pH range in which an aquaculture-recycling system normally operates is between 6 and 8. The concentration of trace metals in this type of system needs to be low because of the high toxicity to fish. Gramm-Osipov *et al.* (1991) reports that a γ-MnO_2 suspension in pure water releases Mn ion at pH = 5.99 (Tab.1). Although those experiments were conducted over a treatment time of 3 days using a micro-suspension of synthetic manganese, which has a greater surface contact than the MnO_2 ore used in this investigation, it is possible that at the lower pH range found in aquaculture-recycling system MnO_2 will be soluble.

In the aquaculture-recycling context, a variety of organic compounds are expected to come in contact with the MnO_2 used as supporting media in bio-filters. The

interaction between hydrous MnO_2 and organic compounds could cause two main processes:

- Adsorption of soluble organic matter by MnO_2, which could affect manganese dissolution and interactions with other chemical species (Tipping and Heaton, 1983).
- Chemical reduction of Mn (IV) by a number of organic compounds (Stone and Morgan, 1984; Lovley, 1991).

It has been recognized that one of the controlling factors in the adsorption of soluble organic matter by MnO_2 is pH (Tipping and Heaton, 1983). In general, the higher the pH, the lower will be the sorptive capacity of humic substances on MnO_2 surface and therefore the higher will be its stability. However, a number of organic compounds have been found to reduce Mn (IV) at circumneutral pH (Stone and Morgan, 1984; Bermingham, 2001). These evidences suggest that organic compounds found in aquaculture recycle-system may affect the stability of MnO_2 ore.

It is expected that a biological community will be established inside the MnO_2 filter bed in the aquaculture-recycling environment, where a high level of bacteria and nutrients are found. Consequently, a variety of chemoheterotrophic and chemoautotrophic bacteria could affect the solubility of solid MnO_2 through direct and / or indirect reduction (see section 2.4).

Curtailment of the O_2 supply inside the MnO_2 filter bed could also encourage microorganisms that can utilize MnO_2 ore as alternate electron acceptor. The process is likely to occur when the diffusion process within the biofilm thickness limits the

transfer of nutrients in the inner layer closer to the MnO_2 interface (inactive thickness), thus establishing an anaerobic environment near the surface of the media. Alternatively, enzymatic reduction of MnO_2 could occur in conditions of partial clogging, and channeling of water flow through the filter bed.

Any bacteria activity lowering the pH and / or redox potential can increasingly shift the Mn (II) – Mn (IV) thermodynamic equilibrium in favour of Mn (II). Consequently, considering the conversion of carbonaceous organic matter and ammonia carried out in biological filters by chemoheterotrophic and chemoautotrophic bacteria, the following chemical reactions could cause a nonenzymatic reduction of MnO_2 ore (Water Pollution Control Federation, 1983; Metcalf & Eddy, 1991):

$$NH_4^+ + 1.5\,O_2 \leftrightarrow 2H^+ + H_2O + NO_2^-$$

$$COHNS + O_2 + \text{nutrients} \rightarrow CO_2 + NH_3 + C_5H_7NO_2 + \text{other end products}$$

The first reaction produces hydrogen ions and as a result lowers the pH in proximity of the MnO_2 interface. Similarly, the conversion of carbonaceous organic matter (COHNS) into new bacterial cells ($C_5H_7NO_2$), while not producing H^+ directly, produces CO_2, which lowers the pH as a result of the following reaction with water:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

Given that organic matter could affect manganese solubility, a nonenzymatic reduction of MnO_2 could also occur because nitrite can reach higher concentration in aquaculture recycling systems compared with the natural environment. Thus, if nitrite can reduce Mn (IV) (Bartlett, 1981) to soluble Mn (II) then a certain degree of solid MnO_2 solubilization could occur inside the filter bed as a direct result of the inlet nitrite concentration or from the biological oxidation of ammonia to nitrite occurring inside the filter bed.

The physical and chemical characteristics of MnO_2 including redox chemistry of manganese and nitrogen, has been described above. Potential microbiological interactions, which appear to occur in water process system as well as in natural environments, have also been highlighted in order to have a complete characterization of the manganese material related to the aquatic environment. Investigation of MnO_2 as a potential carrying media in bio-filters is justified on the bases of the following factors:

1) Specific Surface Area

The capacity of ammonia and nitrite oxidation by microorganisms in a bio-filter depends in part on the number of nitrifying bacteria present in the bio-filter, which is associated with the surface area available for their adhesion (Horowitz and Horowitz, 2000). Because of the high specific surface area reported in literature for MnO_2, it is possible that MnO_2 might have an additional internal surface area (inner surface which extended below the surface topography of the media), available to bacteria, compared to other granular media used as a carrying media in bio-filters.

However, the high surface may not be effective because of:

- Blocking of access to the internal surfaces due to bio-fouling
- The possibility that internal pores size will be too small for bacteria
- Limited water flow through the internal pores being insufficient to provide oxygen and nutrient requirements of the bacteria

Thus, the deposition of organic particles and bacterial growth on the surface of the grain of the filter medium may soon restrict access to the internal surfaces. The adhesion of bacteria will then be restricted to the external surface of the grain. The grade size of the granular media will then be more representative of the actual surface available for bacterial adhesion. The primary cause of this effect is the diffusion-limited movement of nutrient and oxygen inside the biological film zones. As a result a deep inactive mass zone is generated where metabolite removal rate is independent of film thickness (Haug and McCarty, 1972). However, if the dissolution of MnO_2 ore occurs inside the bio-filters, then the MnO_2 could have the following advantages compared with other granular media used as a carrying media in bio-filters:

- Constant regeneration of the interface MnO_2 / biofilm that could contribute to maintain a thin and fully active biomass, particularly without the presence of an 'inactive thickness' biofilm zone.
- The dissolution of MnO_2 ore could increase the size of the internal surface available to bacteria.

As a result of MnO_2 dissolution, a continuous vertical movement of manganese could occur inside the bio-filter bed. For example, in the case of submerged biofilters where the water flows from the top to the bottom, MnO_2 would be reduced in the top level.

The soluble manganese would then be re-adsorbed and oxidized in the lower level of the filter bed by chemical equilibria associated with MnO_2 / Mn^{2+} and / or by biological oxidation. At the same time, a filter backwashing routine should constantly re-distribute manganese throughout the filter bed.

2) Sorptive characteristic

Depending on each type of media's adsorptive capacity, the filter media provides bacteria with varying amounts of nutrients from raw water. Various investigations on hydrous MnO_2 show that in the normal pH range of natural water the surface charge of the material is negative. However, the sorptive characteristic of the MnO_2 system is determined by the charge characteristic of the hydrous oxides which in turn is strongly dependent not only upon the concentration of H^+ but also on the concentration of polyvalent cations. The cation exchange capacity of the hydrous oxides is strongly pH-dependent and an increase with increasing pH (increasing negative charge) and the affinity of the MnO_2 for H^+ and multi-valent cations is larger than for mono-valent alkali ions.

No information has been found in the literature about the sorption of ammonia, nitrite or any intermediate compounds by MnO_2. Considering that the surface charge of the material is negative in the pH range found in aquaculture, it would be expected that ammonia, NH_4^+, is more likely to interact with MnO_2 by electrostatic attraction than nitrite, NO_2^-. The sorptive properties of MnO_2 could favour nitrifying bacterial colonization by providing a constant substrate to feed on. However, MnO_2 is also able to adsorb organic substances, affecting interactions with other chemical species and

favouring heterotrophic bacteria, which are the main competitors of nitrifying bacteria. Finally, the sorption properties of MnO_2 could be modified, or even negated, by the formation of a biofilm on the surface of the media, acting as a physical barrier to metabolite and organic substance attachment to MnO_2.

3) Redox chemistry

A thermodynamic argument exists that a certain degree of Mn (IV) reduction could enhance or facilitate the biological process of ammonia oxidation to nitrate. The process could involve ammonia, but is more likely to affect the hydroxylamine and nitrite, which could potentially reduce Mn (IV). There is also evidence suggesting that nitrite can reduce Mn (IV) in the natural environment (Bartlett, 1981).

Hydroxylamine (NH_2OH) has been recognized as a logical intermediate, possibly the first step, on the way to nitrate production (Hagopian and Riley, 1998). A strong thermodynamic argument applies to the formation of hydroxylamine from the MnO_2 /ammonia reaction:

$MnO_2 \rightarrow Mn^{2+}$	+ 1.22 V
$NH_2OH \rightarrow NH_4OH$	- 0.42 V
$MnO_2 + NH_4OH \rightarrow Mn^{2+} + NH_2OH$	**+ 1.64 V**

Experimental evidences exist, showing that hydroxylamine is nitrified more rapidly than ammonia (Anderson, 1964). More recently, Bock *et al.* (1991) and Frijlink *et al.*

(1992) suggested that, although toxic a low concentration, hydroxylamine can serve directly as a substrate for ammonia-oxidizing bacteria.

4) Bacterial competition in the biological filters

Biological manganese oxidation is exploited in water treatment for Fe (II) and Mn (II) removal, where native water bacteria (e.g. *Leptothrix*) develop and precipitate manganese. *Leptothrix* was found to be the dominant bacteria on sand filters (Xambeau, 1990). When MnO_2 is used as a supporting media in bio-filter, particularly if the reduction of manganese occurs inside the filter bed, it is likely that a different biological community will develop compared with conventional bio-filters. The Mn oxidizing bacteria could become dominant and compete with nitrifying bacteria and heterotrophic bacteria for surface space and also for oxygen up-take. As a result, this condition could also modify the competitive relation between nitrifying bacteria and heterotrophic bacteria. Overall, a different metabolite removal performance could be obtained from MnO_2 bio-filter because a different biological community is established.

To summarize, the investigation of MnO_2 as a supporting media for bio-filters appear to be intricate. Physical, chemical and biological factors, singularly and/or interrelated, could potentially affect manganese solubility and the performance of MnO_2 in terms of metabolite removal. For this reason the present investigation adopted the following sequence of work:

1) Surface area measurements and surface morphology analysis were carried out in order to compare the surface area available to bacteria of MnO_2 with different types of commercial bio-media.

2) A set of lab experiments, in the absence of biological activity, was carried out with commercial MnO_2 and simulated effluent solutions to determine the ammonia and nitrite adsorption and compare the reactions of MnO_2 against those of silicate sand media.

3) Two pilot-scale pressurised filters were installed in a commercial fish farm in order to compare metabolite removal performance in production conditions between silicate sand and pure MnO_2 media in presence of biological activity.

The media surface studies (1) and the lab experiments (2) methods and results are presented in the next chapter, while the pilot-scale trials (3) procedures and results are described in the following chapter.

Chapter 3
Laboratory trials

3.1 Introduction

Although MnO_2 is one of the favoured materials in water filtration treatments for iron and manganese removal by chemical means, it does not appear to have been considered as a carrying media in biological treatments. As previously discussed, this investigation appears to be complex because physical, chemical and biological factors, singularly and/or interrelated, are involved in manganese solubility and in metabolite removal. Consequently, only physical and chemical aspects were initially considered, while biological factors were separately investigated in the second part of this study. Hence, the suitability of MnO_2 ore as a bio-filter media was firstly investigated in laboratory by studying:

- Specific surface area and surface morphology
- MnO_2 solubility
- NH_4^+/NO_2^- adsorption on MnO_2
- NH_4^+/NO_2^- reaction with MnO_2

Specific surface area and surface morphology:

Synthetic samples of MnO_2 exhibit a high specific surface area (Morgan and Stumm, 1965; Murray, 1973; Balistrieri and Murray, 1982), which could provide a large surface area for the attachment of a great number of bacteria. As an initial step into the investigation of MnO_2 as a supporting media in bio-filters, this research focused on the specific surface area (SSA) of different commercial granular media used as

biological carriers of fixed bio films. The aim was to compare the specific surface area of MnO_2 with different types of commercial bio-media commonly used in the aquarium and aquaculture sector.

The following commercial bio-media were tested and compared with manganese dioxide:

1. Volcanic rock (VR)
2. Sintered glass (SG)
3. Granular activated carbon (GAC)
4. Silicate sand (SS)

VR and SG had been chosen because of a high available surface area is claimed by the supplier. GAC was tested because in many industrial applications it has been shown to have a very high specific surface area, for example, Buzio and Alberti (1995) reported a surface area of 1250 $m^2 g^{-1}$. SS represents the most commonly used granular media for biofiltration treatment in intensive aquaculture system. Thus, SS will be used as the main comparative material during this study on MnO_2.

In order to obtain information concerning factors that affect the ability of a filter medium to function as an anchor for bacteria (Spotte, 1979), the surface morphology of each commercial granular media was examined by scanning electron microscopy (SEM). The SEM studies aimed to give an idea of the roughness of the particle surface and a qualitative estimation of the actual surface area available to bacteria, which could be related to the specific surface area initially measured.

Manganese ore solubility:

The primary concern about the application of MnO_2 in an aquaculture context is represented by the high toxicity of manganese to fish (Exley and Phillips, 1988). MnO_2 is significantly more soluble than thermodynamics would suggest (Savenko, 1985; Swain *et al.*, 1975; Gramm-Osipov *et al.*, 1991). Most experimental evidence is based on ultra-pure, micro-suspension of synthetic MnO_2; for example Gramm-Osipov *et al.* (1991) reports that γ- MnO_2 suspension releases Mn ion at pH = 5.99.

However, this research examines the application of MnO_2 in the aquaculture context, using crushed natural ore graded at suitable particulate size for the application in filtration treatment. Bermingham (2001) studied the solubility of MnO_2 as a function of pH and solution type. Equilibration experiments in distilled water showed I) an inverse relationship between pH and conductivity of the solution, II) that pH dropped with contact time, III) the concentration of 'soluble' manganese increased with contact time. Bermingham (2001) noted that at around pH 4.5 the $[Mn_{sol}]$ increased drastically. In a second set of experiments in a solution of 0.1M $NaClO_4$, used to maintain the ionic strength of the solution, the author showed an inverse relationship between pH and MnO_2 solubility where a marked increase in $[Mn_{sol}]$ was observed around pH 5.

As an initial step in the investigation on MnO_2 as a supporting media in bio-filters, a set of experiments was carried out with commercial MnO_2 and pure water to determine the position of equilibrium between MnO_2 and soluble manganese.

Chemical action of manganese dioxide on metabolites:

The investigation on MnO_2 as a supporting media in bio-filters was initially justified on the basis of its potential sorptive characteristic and redox chemistry with respect to NH_4^+ and NO_2^-. No information, however, has been found in the literature concerning the sorption of NH_4^+, NO_2^- or any intermediate oxidation compounds by MnO_2. A thermodynamic argument can be made that a certain degree of Mn (IV) reduction could enhance or facilitate the biological oxidation of NH_4^+ oxidation to nitrate (NO_3^-). The process could involve NH_4^+, but is more likely to implicate the hydroxylamine (NH_2OH) and NO_2^-, which could potentially reduce Mn (IV) (Bartlett, 1981). A set of experiments was carried out with commercial MnO_2 to determine the extent of NH_4^+ and NO_2^- adsorption, the degree, if any, of oxidation and to compare the sorption/oxidation characteristics of MnO_2 against those of silicate sand media. These tests were carried out on two reaction scales, batch trials and pilot trials.

Batch trials were the first step into the investigation of MnO_2 as a biological media. Preliminary tests involved a simple experimental set-up with static testing conditions to examine the effect of contact between metabolite solution and MnO_2 ore. The aim was to maintain simple water chemistry conditions in order to highlight specific chemical interactions related to MnO_2 and metabolites. Batch trials were carried out with MnO_2 since most of the chemical properties of MnO_2 found in literature used micro-suspension of synthetic MnO_2.

Pilot columns were used to see if the results obtained with static batch test conditions were replicable on a larger scale under dynamic flow. From the outcome of the batch

results, these trials focused on the chemical reaction between NO_2^- and MnO_2. This interaction was studied to see if MnO_2 could perform similar oxidation processes under conditions where the NO_2^- solution flowed through the media. The pilot column was tested continuously for one week in order to determine the affect of prolonged contact with MnO_2 on the NO_2^-oxidation reaction. In addition, the investigation aimed to establish if the chemical make-up of water in the standard solution would effect the NO_2^- reaction with MnO_2. As a result, different types of reconstituted fresh water were used to test this.

3.2 Materials and methods

3.2.1 Specific surface area developed on different commercial granular media

Table 2 summarises the types, dimensions and sourcing of the granular media used in the batch trials

Media type	Grade size (mm)	Producers / Suppliers
VR (EHFILAV)	10 - 5	Animal House Ltd.
SG (EHFISUBSTRAT)	10 - 5	Animal House Ltd.
GAC (API CARBONS)	3 - 2	Animal House Ltd.
MnO_2	0.850 – 0.355	Fergusson and Wild & Co Ltd.
SS	1.19 – 0.595	Fergusson and Wild & Co Ltd.

Table 2: Samples of VR, SG, GAC, SS and MnO_2 obtained from commercial producers/suppliers.

To obtain comparable particle sizes and reduce the affect of particle size on surface area, samples of each media were filtered through sieves and the particle fraction of diameter between 0.5-0.6 mm collected. The isolated sample was re-sieved and the 0.5-0.6 mm fraction again collected. The dry-density of each of the media was recorded using this particle-size range to fill a volume of 10 cm^3. Close packing of particles was achieved by agitation during and after filling. The mass of each 10 cm^3 sample was recorded. The packing process was repeated three times and the mass recorded, to produce the average dry-density value for each of the four media. This dry-density will however include a component of mass for adsorbed water, *etc.*

Desorption experiments (Appendix A) showed that after heating, the average weight loss percentage for the different media was respectively:

1. SG: 1.25%
2. SS: 0.81%
3. VR: 6.26%
4. MnO_2 : 2.27%
5. GAC: 7.69%

The surface area was determined at the Mitsui-Babcock Technology Centre, Paisley, Scotland on a Micromeritics Gemini II surface analyser. Samples were heated for 2 hrs at T>373 K under a dry nitrogen stream to remove adsorbed water etc., then capped and cooled under a steam of dry nitrogen before analysis using He gas as the adsorbent. The measurement of the surface area was determined by the adsorption of helium onto a known quantity of each material and the results modelled using the Brunauer-Emmett-Teller (BET) adsorption isotherm (Appendix A).

3.2.2 Surface morphology observations

The four different commercial media samples tested for specific surface area were then used for SEM studies and compared with MnO_2. This meant that particles were of a comparable grade size. 50 cm^3 of media was air-dried at room temperature in the fume cupboard and about 50 particles from each media were mounted on supporting stubs and sputter coated with gold in an argon atmosphere at vacuum pressure of 3 x 10^{-1} mbar. A Philips (FEI) 500 Scanning Electron Microscope was used to observe the surface morphology of single particles from the five different types of media. An average of 40 particles per media type was observed at high magnification (x2500), at relatively low magnification (x320) and at very low magnification (x40).

3.2.3 Manganese ore solubility and chemical action of silicate sand and manganese dioxide on ammonia and nitrite

Equipment:

pH was measured with a 'Philips – PW 9409 digital pH-meter calibrated daily with buffer solutions of pH 4 and pH 7. Dissolved oxygen was measured with 'YSI model 58' DO meter. A 'UNICAM 939' AA spectrometer equipped with a 'UNICAM GF 90' furnace was used for the quantitative analysis of total Mn and [Mn_{sol}].

Samples for vacuum extractions for quantitative analysis of [Mn_{sol}] were filtered by Whatman GF/C 47 mm followed by a final filtration with Whatman Cellulose Nitrate membrane 0.22 μm filter papers. For the purpose of this experiment, soluble

manganese is therefore defined as that component of the solution that can pass through a 0.22 μm filter paper.

Materials and methods used for the measurement of ammonia, nitrite and nitrate are described in Appendix B (Golterman *et al.*, 1978 in Stirling, 1985)

Materials:

SS and MnO_2 used in batch and pilot trials were obtained from Universal Mineral Supplies (UMS) Ltd. The sand had a diameter range of 1.19 mm – 0.595 mm (16/30 grade size). The MnO_2 ore had a diameter of 0.850 mm – 0.355 mm (18/44 grade size) and is listed as having a manganese content of 70-75% as MnO_2. The diameter range selected is crucial to optimum blending with sand to maximize the distribution of the ore in the water filtration process (Briggs, 1980).

Bermingham (2001) carried out elemental analysis of natural MnO_2 ores comparing two different sources of manganese dioxide obtained from Universal Mineral Supplies (UMS) Ltd and Fergusson Wild & Co Ltd. These results showed that manganese content was very similar between the two MnO_2 ores, but there were variations in the magnitude of other components. Thus, values for Fe, Mg, Ca and Na for Fergusson Wild & Co Ltd MnO_2 were notably higher than Universal Mineral Supplies (UMS) Ltd MnO_2 ore (Table 3). These variations between the two natural ores are probably the result of the prevailing geochemical cycle within the different formation area from which the ore was extracted (Bermingham, 2001).

Sample	1	2	3	**Average**	4	5	6	**Average**
Conc. **Mn** (g/L)	0.0612	0.0462	0.0487	**0.0520**	0.0465	0.0551	0.0494	**0.0503**
Mn%	47.08	47.63	45.51	**46.74**	44.71	48.33	45.74	**46.26**
MnO2%	74.47	75.34	71.99	**73.93**	70.73	76.45	72.35	**73.18**
Conc. **Fe** (mg/L)	4.780	3.643	4.025	**4.149**	7.710	6.492	6.381	**6.861**
Fe%	3.68	3.76	3.76	**3.73**	7.41	5.69	5.91	**6.34**
Conc. **Ca** (mg/L)	0.079	0.064	0.066	**0.070**	0.236	0.189	0.199	**0.208**
Ca%	0.06	0.07	0.06	**0.06**	0.23	0.17	0.18	**0.19**
Conc. **K** (mg/L)	0.902	0.681	0.910	**0.831**	0.661	0.905	0.772	**0.779**
K%	0.690	0.700	0.850	**0.747**	0.640	0.790	0.710	**0.713**
Conc. **Mg** (mg/L)	0.141	0.105	0.120	**0.122**	0.200	0.229	0.232	**0.220**
Mg%	0.11	0.11	0.11	**0.11**	0.19	0.20	0.21	**0.20**
Conc. **Na** (mg/L)	0.442	0.441	0.347	**0.410**	3.856	1.882	1.128	**2.289**
Na%	0.34	0.45	0.32	**0.37**	3.71	1.65	1.04	**2.13**

Table 3: Elemental Analysis of Natural Manganese Dioxide Ores (Bermingham, 2001). Samples 1, 2 and 3 originated from one ore (Universal Mineral Supplies Ltd) and samples 4, 5 and 6 from another source (Fergusson Wild & Co Ltd).

Ultra-pure double distilled nitric acid 99.99% (Fisher) was used to acidify samples extracted for the quantitative analysis of Mn_{sol}. Samples were refrigerated until analysed.

Standard solutions were made from double distilled water (DD) and sodium bicarbonate (30 mg L^{-1} of HCO_3^- as $NaHCO_3$), which was used to adjust the pH of the solution in a range between 7.03 and 7.13. Different metabolite test solutions were made by adding ammonium chloride (NH_4Cl), sodium nitrite ($NaNO_2$) and sodium nitrate ($NaNO_3$) to the bicarbonate stock solution. The following metabolite solution were tested:

- 5 and 10 mg L^{-1} of NH_4-N as NH_4Cl
- 5 and 10 mg L^{-1} of NO_2-N as $NaNO_2$

- 10 mg L^{-1} of NO_3-N as $NaNO_3$

Synthetic hard and soft water solutions were made up according to the compositions listed in Table 4 (American Public Health Association, 1989) using compounds supplied by Aldrich Chemical Company (Aldrich).

	Hard Water	**Soft Water**
Compounds	mg L^{-1}	mg L^{-1}
$NaHCO_3$	192	48
$CaSO_4$	120	30
$MgSO_4$	120	30
KCl	8	2

Table 4: Composition of Synthetic Hard and Soft Water Solutions

The manganese solubility and the effect of adsorption and reaction in the removal of ammonia and nitrite were assessed on 'new' media taken from sealed bags and therefore considered 'free' from bacterial contamination. In order to remove Mn 'dust', each 'new' media batch was pre-rinsed with 20 L of DD water. Experiments were carried out using 100% silicate sand as a control against a standard mixture of sand (80%) and MnO_2 (20%). Silicate sand was used as a control because it is commonly used in biological filters and because sand is combined with MnO_2 in water treatment systems for iron and manganese removal.

3.2.4 Batch trial experimental procedures

The control and standard batches were made respectively of pure sand and sand mixed with MnO_2 ore (20% by volume). Figure 5 shows a schematic diagram of the equipment used for the batch tests.

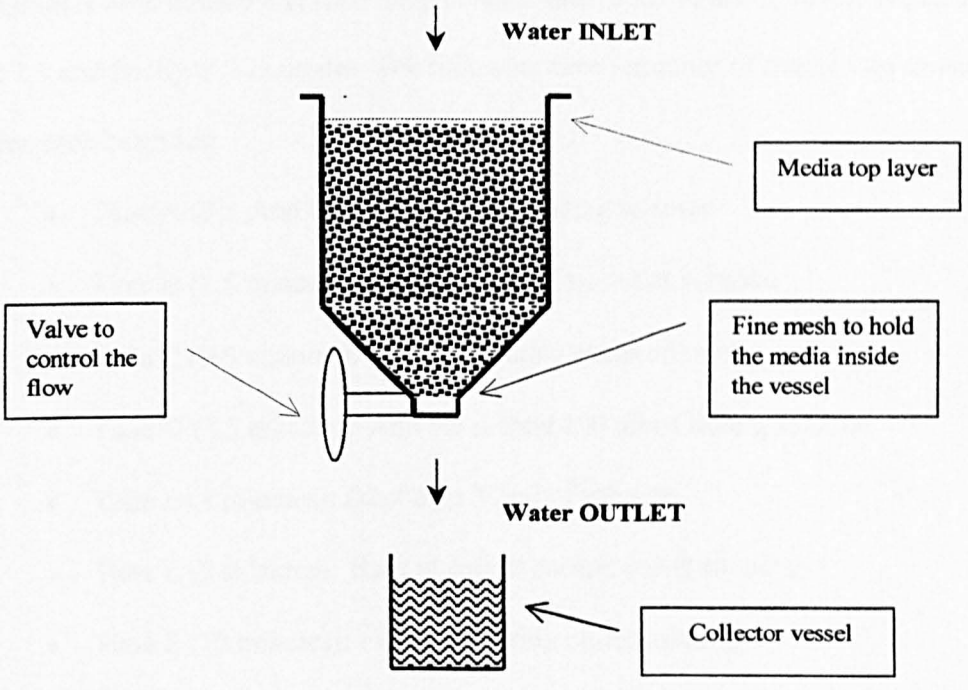


Figure 5: Equipment used for the batch tests.

The rinsed material was placed inside the batch vessel (Figure 5), and added in such a way as to ensure that the media was uniformly packed. An initial rinse with 150 ml standard solution was made in order to remove any residual 'dust' created as a result of setting up the experiment. For each test, 100 ml of the test solution was used to saturate the void space created by the media inside the vessel. Two replicates were made for each batch trial in this set of experiments.

Manganese solubility batch trials:

Standard batches, containing 748g ± 1 of sand and 178g ± 1 of MnO_2, were initially used to test manganese solubility as a function of pH. DD water at pH of 5.7 and standard solutions at pH 7.5 were tested and the equilibrium between soluble manganese (Mn^{2+}) and MnO_2 was observed over a 20-minute period of contact where samples were collected at increasing contact time of 2.5 minutes, 5, 7.5, 10, 12.5, 15, 17.5 and finally at 20 minutes. The following time sequence of events was carried out for each batch test:

- Time A (0): Add the first 100 ml of testing solution
- Time B (2.5 minutes): Start to collect first outlet solution
- Time C (3.5 minutes): Finish collecting outlet solution
- Time D (3.5 minutes): Add the second 100 ml of testing solution
- Time E (4 minutes): Discharge 30 ml of solution
- Time E (9 minutes): Start to collect second outlet solution
- Time F (10 minutes): Finish collecting outlet solution

The procedure was repeated in this manner until a 'contact time' of 20 minutes was reached. The purpose of the solution discharge (Time E) was to remove any residual solution from the previous contact time test.

For these experiments, the following water parameters were measured for the inlet and outlet water solutions from the batch trials:

- Temperature
- pH
- Dissolved oxygen

- Total and Soluble manganese (Mn_{sol}) concentration

Metabolite adsorption and reaction batch trials:

A control batch was made using 2100g ±1 of 16/30 sand. The 'standard' batch consisted of 1870g ±1 of 16/30 sand mixed with 444g ±1 of 18/44 MnO_2. These values ensured that the same volume of media (1.37 L) was maintained between both types of batches. A greater volume of media, compared with the first set of tests on manganese solubility, was required because a certain volume of outlet solution was needed in order to carry out additional analyses (see Appendix B).

Standard batches were compared to control batches to test adsorption and reaction of metabolite from both MnO_2 and sand. During these trials, the manganese solubility of the standard batch was also considered. A standard solution with pH range between 7.03 and 7.13 was tested at the following metabolite concentration:

- 5 and 10 mg L^{-1} of NH_4-N as NH_4Cl
- 5 and 10 mg L^{-1} of NO_2-N as $NaNO_2$
- 10 mg L^{-1} of NO_3-N as $NaNO_3$

The following sequence was carried out for each batch test:

Testing day 1

- Time A (0): Add the first 100 ml of metabolite solution
- Time B (5 minutes): Start collecting first outlet solution
- Time C (6 minutes): Finish collecting outlet solution
- Time D (9 minutes): Add the second 100 ml of metabolite solution

- Time E (14 minutes): Start collecting second outlet solution
- Time F (15 minutes): Finish collecting outlet solution

Testing day 2

- Time G (24 hours): Add the first 100 ml of standard solution
- Time H (24 hours and 5 minutes): Start collecting outlet solution
- Time I (24 hours and 6 minutes): Finish collecting outlet solution
- Time J (24 hours and 9 minutes): Add the second 100 ml of standard solution
- Time K (24 hours and 14 minutes): Start collecting second outlet solution
- Time L (24 hours and 15 minutes): Finish collecting outlet solution

Between time C and D; F and G; I and J the batch was left to drain under gravity. A contact time of 5 minutes was chosen for the initial trials and compared with a second test involving a longer contact time of 24 hours in order to maximize the time for oxidation of metabolite adsorbed on the surface of MnO_2. The 5-minute contact time was selected because this approximates to the contact time commonly found inside submerged granular biological filters.

For these experiments, the following water parameters were measured for the inlet and outlet water solutions from the batch trials:

- Temperature
- pH
- Dissolved oxygen
- Ammonia, nitrite and nitrate
- Soluble manganese (Mn_{sol})

3.2.5 Pilot trial experimental procedure

A mini-recirculation system was created by pumping the water from the aquarium into the top of the column (inlet), which drained through the media under gravity returning to the tank via the bottom of the column (outlet) (Figure 6). Table 5 lists the basic technical characteristics of this small closed system.

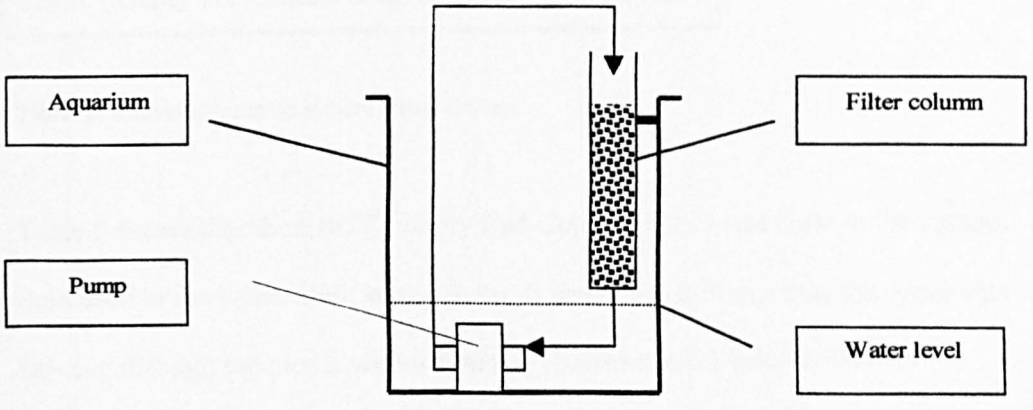


Figure 6: Mini-recirculation system layout.

Each pilot trial consisted of a plastic column (length = 80 cm, diameter = 5 cm, volume = 1.57 l) installed on a 20 L glass tank operating at 25 °C. The plastic column was filled with 1870g ±1 of 16/30 sand mixed with 444g ±1 of 18/44 MnO_2 (same volume, grade size and composition of standard batches). The media was pre-rinsed with 20 L of DD water in order to remove any dust. It was then placed inside the plastic column, ensuring that the media was uniformly packed. An initial rinse with 150 ml standard solution was made in order to remove residual dust created as a consequence of the batch set up. At this stage the column was fixed to one side of the glass tank, and plugged to the pump to bring the water to the column.

Aquarium characteristics:	
Aquarium volume (m^3)	0.01
Pilot column flow rate (m^3/hr)	0.015
Aquarium residence time (minutes)	40
Pilot column characteristics:	
Flow rate (m^3/hr)	0.015
Size of the bed (m^2)	0.002
Velocity (m/hr)	7.64
Depth of the bed (m)	0.69
EBCT (Empty bed contact time) (minutes)	5.42

Table 5: Mini-recirculation system technical data.

Table 5 shows that the EBCT (Empty Bed Contact Time) was close to the contact time used in the batch trials, but with the difference that in this case the water was flowing through the media with a dynamic (average bulk) velocity flow of approximately 8 m/h.

The pilot trial was replicated twice, using each time 'new' media, each time at 5 mg L^{-1} concentration of NO_2-N as $NaNO_2$ with the following water solutions:

- Standard solution used for the batch
- Reconstituted soft water
- Reconstituted hard water

Water samples were daily collected for one week. Evaporation from the system was daily replaced by adding fresh water solution. The following water parameters were measured:

- Dissolved Oxygen

- pH
- Nitrite and nitrate
- Total Mn and [Mn_{sol}]

3.3 Results

3.3.1 Specific surface area developed by different commercial granular media

Table 6 shows the results of the surface area measurements.

Sample	Medium	m^2/g	m^2/L
1	Sintered glass**	0.26*	260
2	Silicate sand**	0.28*	470
3	Volcanic rock**	0.85*	560
4	MnO_2**	14.25	27,870
5	MnO_2***	14.30	27,340
6	Activated carbon	711.84	371,580

**It should be noted that, because of the experimental limitations, values of less than $1 m^2 g^{-1}$ should be viewed with caution as these values are around the limit of the instrument sensitivity (McLaughlin, personal communication).*

*** Particle range (0.6mm – 0.5 mm)*

****Particle range (0.850mm – 0.355mm)*

Table 6: Surface Area (BET) measurements on filter media.

The surface area (BET) measurements on filter media were expressed in terms of surface area per unit of mass ($m^2 g^{-1}$). By knowing the dry-packed density of the different filter media (Appendix A), it was possible to determine a measure of the

surface area developed per unit of volume ($m^2 L^{-1}$). Specific surface area of a media per unit of volume is important as the cost of building a bio-filter is directly proportional to its size and filter size is directly related to the volume of media required.

These results can be summarized as follows:

- The GAC has a significantly greater surface area per particle than any other media tested
- MnO_2 has a significantly greater surface area (approximately 10 times greater) than SG, SS and VR
- A particle size effect is not observed for MnO_2, as the sample with defined particle range (0.6 mm - 0.5 mm) shows no major difference in surface area from the wider grade range (0.850 mm – 0.355 mm) sample.
- SG, SS and VR have surface areas of a similar order of magnitude, although results suggest that VR is slightly higher

The results obtained from the two different grade of MnO_2 would suggest that the inner surface area is much greater compared with the external surface area. Thus, for MnO_2, up to a certain extent the SSA appear to be independent from particle size.

The commercial sector often approximates the effectiveness of bio-filter media by surface area, as determined by BET or other adsorption methods (Horowitz and Horowitz, 2000). The basic supposition behind this approach is that media with high surface areas will support greater numbers of bacteria. The validity of this method relies upon the assumption that the interaction of the bacteria with the filter media is

similar to that of the inert gas used in the adsorption experiments. On the basis of size exclusion, this seems unlikely, since the atomic and molecular dimensions of He and N_2 are of the order of 2×10^{-10} m (Housecroft and Constable, 2002) while bacterial dimensions lie in the range 2×10^{-6} m (Watson, 1971; Bock *et al.*, 1989). As a result the gases used in BET can penetrate the inner surface below the surface topography of the medium, which bacteria may be unlikely to negotiate. It would appear that the use of surface area determined by adsorption methods is too simplistic approach when evaluating bio-filter media. Nevertheless by combining surface area data with surface morphology observations it could be possible to determine more realistically the suitability of a medium for sustaining a bacteria population.

3.3.2 Scanning electron microscopy images

The BET results obtained from the MnO_2 suggest that the inner surface which extended below the surface topography of the media appears to be complex. Thus, it is difficult to determine 3D media spaces from the micrographs obtained from the SEM. As a result only indirect impressions could be gathered by observing the areas of shadow formed on the surface of the medium.

The following morphological elements were evaluated during this study:

- Media grain shape
- Concave surface in light with area greater than 1000 μm^2 were defined as creeks likely to be fully colonised by bacteria.
- Surfaces in shadow with an area greater than 500 μm^2 were defined as cavities likely to be partially colonized by bacteria.

- Surfaces in shadow with an area smaller than 500 μm^2 were defined as pores unlikely to be colonized by bacteria but probably able to provide a particularly high surface area for the attachment of single bacteria cell.

Micrograph studies at magnification Macro x 40

- The grains of SG, VR, GAC and MnO_2 were of angular shape (Figure 7, 8, 9 and 10) at this low magnification
- The grains of silicate sand appeared to have a more rounded and smoother shape (Figure 11)

Micrograph studies at magnification Macro x 320

- The surface of the SG, VR and GAC exhibit complex surface morphology with numerous creeks and cavities (Figures 12, 13 and 14).
- The surface of SS and MnO_2 appeared simple with a limited numbers of creeks and cavities (Figures 15 and 16).

Micrograph studies at magnification Macro x 2500

- At high magnification, the 'micro-surface' of the SG was relatively smooth (Figure 17); GAC and VR have complex microstructure with numerous small cavities (Figure 18 and 19).
- The surfaces of SS and MnO_2 exhibit a simple microstructure but have a degree of porosity (Figures 20 and 21). The extent of the surface porosity appeared to be much greater for MnO_2 than for sand.

Figure 7: SEM observation of sintered glass (SG) grains, magnitude = 40. One bar represents 100 μm.

Figure 8: SEM observation of volcanic rock (VR) grains, magnitude = 40. One bar represents 100 μm.

Figure 9: SEM observation of granular activated carbon (GAC) grains, magnitude = 40. One bar represents 100 μm.

Figure 10: SEM observation of "MnO_2" grains, magnitude = 40. One bar represents 100 μm.

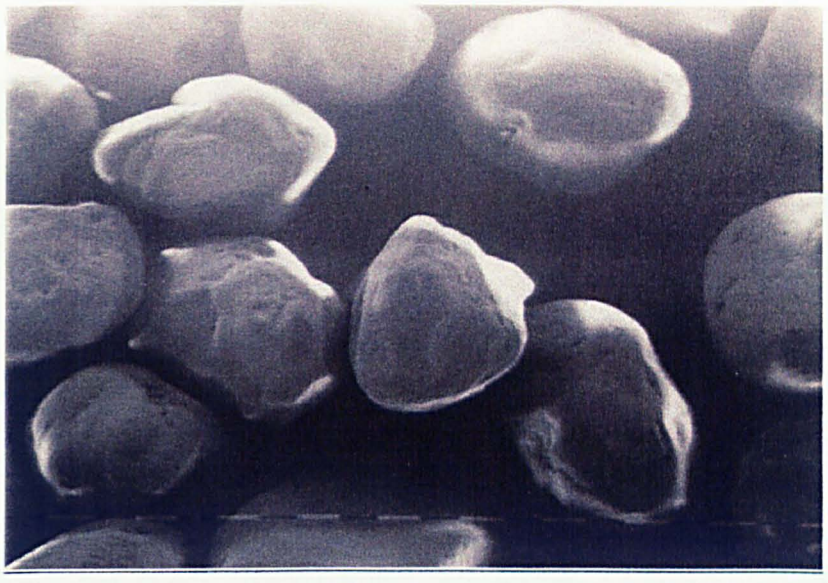


Figure 11: SEM observation of silicate sand (SS) grains, magnitude = 40. One bar represents 100 μm.

Figure 12: SEM observation of a sintered glass (SG) grain, magnitude = 320. One bar represents 10 μm.

Figure 13: SEM observation of a volcanic rock (VR) grain, magnitude = 320. One bar represents 10 μm.

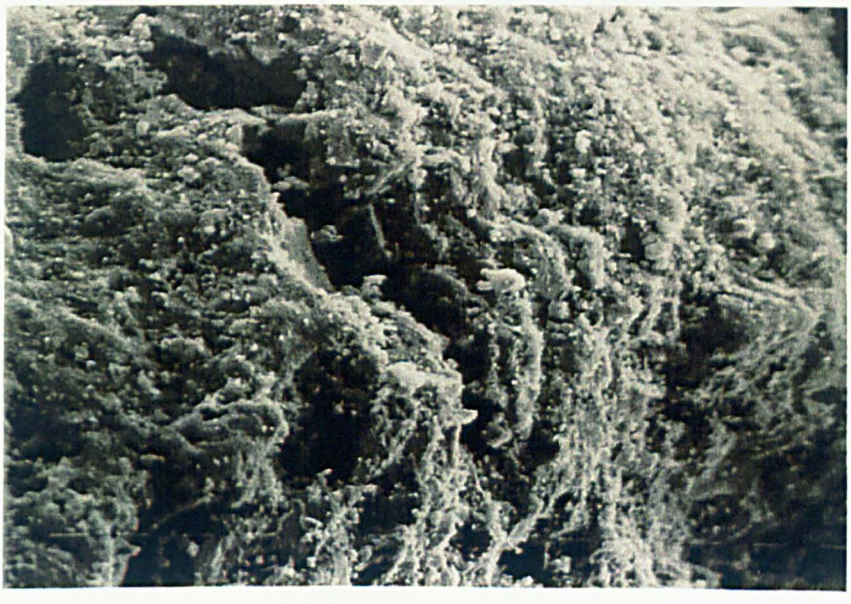


Figure 14: SEM observation of a granular activated carbon (GAC) grain, magnitude = 320. One bar represents 10 μm.

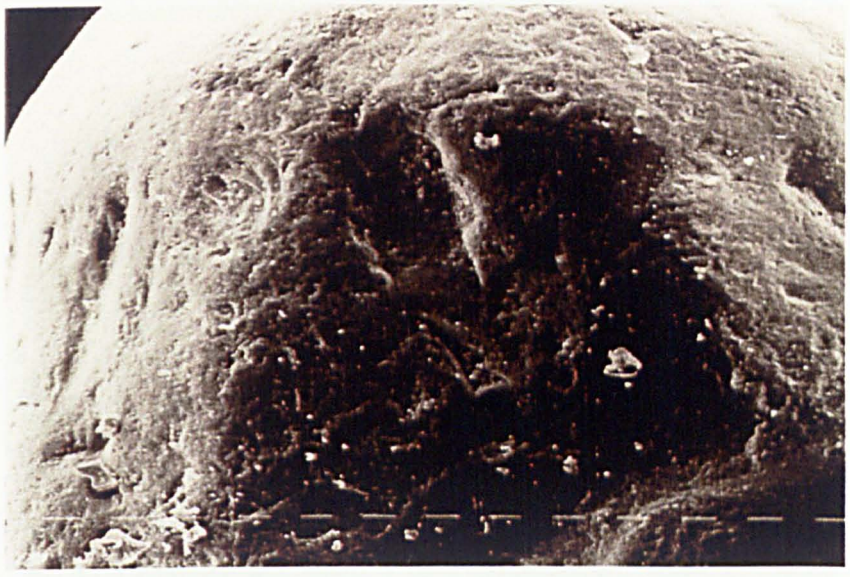


Figure 15: SEM observation of a silicate sand (SS) grain, magnitude = 320. One bar represents 10 μm.

Figure 16: SEM observation of **"MnO_2"** grain, magnitude = 320. One bar represents 10 μm.

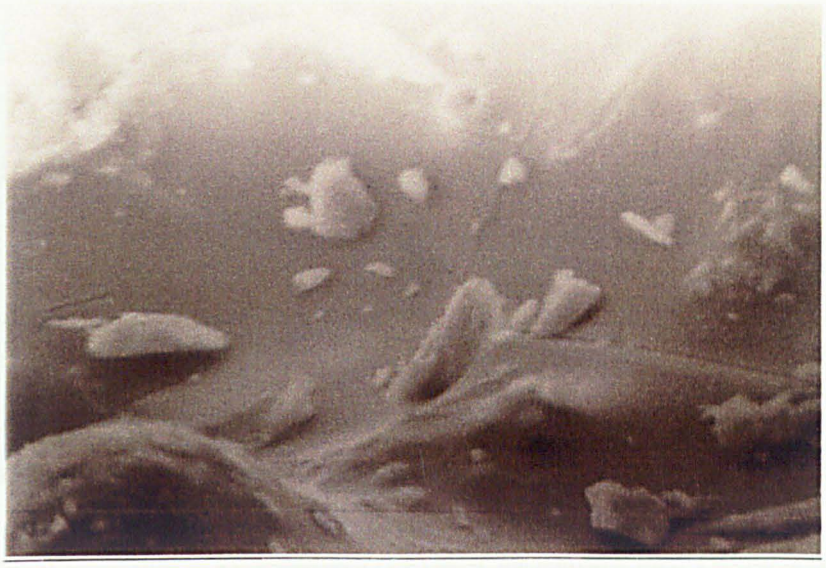


Figure 17: SEM observation of a sintered glass (SG) grain, magnitude = 2500. One bar represents 10 μm.

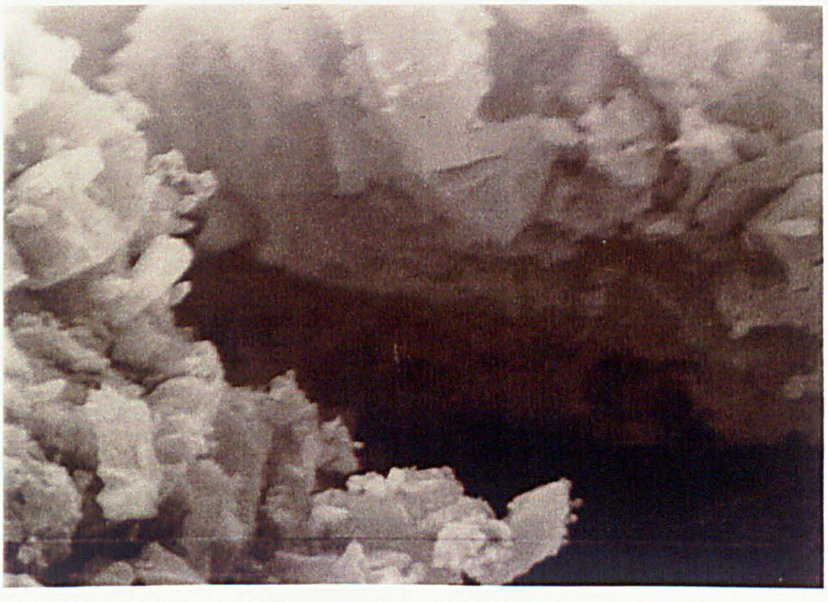


Figure 18: SEM observation of a granular activated carbon (GAC) grain, magnitude = 2500. One bar represents 10 μm.

Figure 19: SEM observation of a volcanic rock (VR) grain, magnitude = 2500. One bar represents 10 μm.

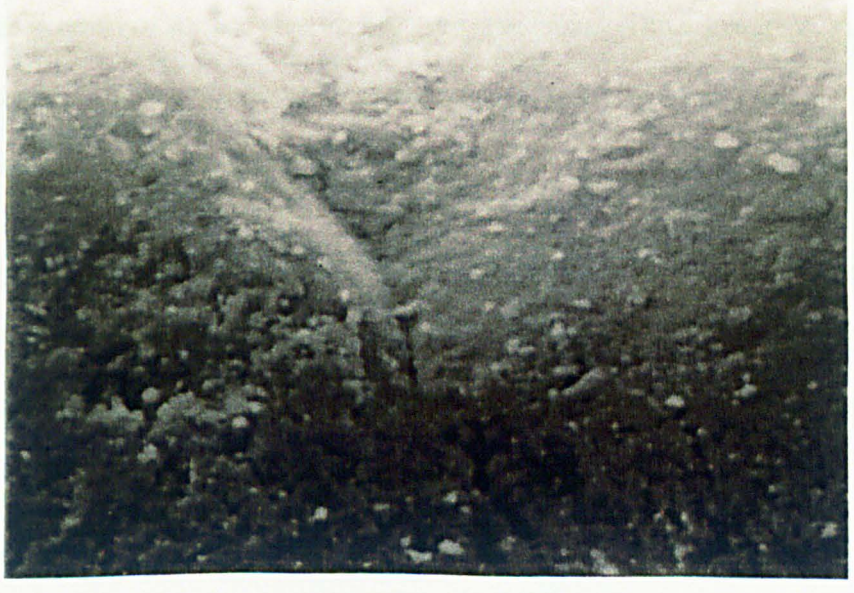


Figure 20: SEM observation of a silicate sand (SS) grain, magnitude = 2500. One bar represents 10 μm.

Figure 21: SEM observation of MnO_2 grain, magnitude = 2500. One bar represents 10 μm.

Spotte (1979) suggests that coarse angular materials are preferable to smooth materials in terms of surface area for bacterial attachment. The micrograph studies at magnification Macro x 40 showed that SG; VR; AC and MnO_2 had a more pronounced angular grain shape compared with SS.

Micrograph studies at magnification Macro x 320 showed that the surface available to bacteria appears to be greater for SG, VR and AC compared with SS and MnO_2. The surface area (BET) measurements showed very different values for SG, VR and SS when compared with MnO_2 and AC. The fact that micrograph studies at magnification Macro x 2500 revealed a complex microstructure for SS and particularly for MnO_2 could explain the high surface area (BET) measurements when compared with the other media.

As discussed above, on the basis of the micrograph observations, it seems likely that high surface area (BET) measurement does not relate directly to surface available to bacteria. As the surface area (BET) measurements and morphological study progressed, the following trend emerged:

The surface area (BET) measurements and micrograph observations carried out on different commercial bio-media suggest that MnO_2 and sand media have lower surface areas available to bacteria than GAC, and comparable surface areas to sintered glass and volcanic rock. GAC has a significantly greater surface area per particle than any of the other media examined. Micrograph observations at medium magnification (Macro x 320), shows the surface of silicate sand and MnO_2 to be simpler, with a limited numbers of creeks and cavities, when compared to GAC, sintered glass and volcanic rock. However, examining the different bio-media at higher magnification (Macro x 2500) indicates that the surface of silicate sand and MnO_2 have a complex microstructure with numerous pores. Such a pore structure is not observed for sintered glass. The extent of the surface porosity appeared to be much greater for MnO_2 than for sand. The dimension of these pores (less than 500 μm^2) was considered too small to provide an extra surface available for bacterial colonisation. Nevertheless, while surface area is important, the surface morphology of sand and in particular of MnO_2 media could provide a better 'anchor' for bacteria cell when compared with GAC, sintered glass and volcanic rock.

3.3.3 Batch trials of manganese ore solubility and chemical action of silicate sand and manganese dioxide on ammonia and nitrite

The results of manganese solubility for the first set of batch trials are shown in Figure 22. The results can be summarized as follows:

- As expected, Mn solubility is pH dependent
- At an inlet pH of 7.5, Mn solubility appears to rapidly establish an equilibrium concentration of about 10μg L^{-1}
- At an inlet pH of 5.7, $[Mn_{sol}]$ is increased and appears to be increasing with time even after 20-minute reaching a concentration of 17.5μg L^{-1}
- In both cases, the $[Mn_{sol}]$ reaches appreciable levels in the first few minutes; *i.e.* < 2.5 min.

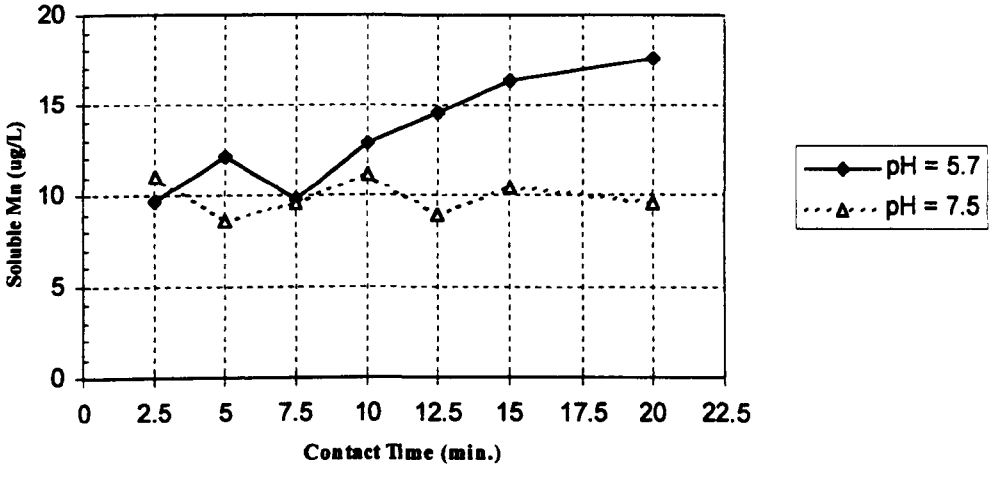


Figure 22: Manganese dioxide solubility as a function of pH.

The results of both comparative metabolite adsorption and reaction trials are based on the higher metabolite concentration tested (10 mg L^{-1} as NH_4-N, NO_2-N and NO_3-N),

and it is the net metabolite mass balance at time L of the batch methodology described in section 3.2.3.1 for the metabolite adsorption and reaction tests. The data collected during these trials are summarized in Appendix C.

Ammonia adsorption:

The results of NH_4^+ adsorption trials are shown in Table 7.

	Control Batches	Test Batches
NH_4-N adsorbed in µg	3,364	3,432
NH_4-N adsorbed per litre of media in µg L^{-1}	2,456	2,505
NH_4-N adsorbed efficiency in %	83.52	87.91
Efficiency of standard batch in comparison to control batch in %		4.39
Average adsorption rate in µg/g/min.	0.16	0.15

Table 7: Results of comparative ammonia adsorption trials. Values are the mean of two replicates.

The calculation for the values showed in Table 7 was carried out as follows (see also appendix C):

1. Total NH_4-N input[1] = ([NH_4-N] Time A + Time D) x Volume solution
2. Total NH_4-N output[2] = [([NH_4-N] Time C + Time F) x Volume solution

[1] *100 ml test solution added during Time A + 100 ml test solution added during Time D = 200 ml solution added.*

[2] *It was assumed that the amount of solution collected at the outlet of the batches was equal to the amount added during Time A + Time D. This was not completely true since a small amount of solution was trapped inside the batch bed.*

3. Total NO_3-N input = ([NO_3-N] Time A + Time D) x Volume solution
4. Total NO_3-N output = [([NO_3-N] Time C + Time F) x Volume solution
5. NH_4-N adsorbed = (Total NH_4-N input - Total NH_4-N output) + (Total NO_3-N output – Total NO_3-N input)
6. NH_4-N adsorbed per litre of media = NH_4-N adsorbed / Batch Volume
7. Adsorption efficiency (%) = NH_4-N adsorbed / NH_4-N input
8. Average adsorption rate (µg/g/min) = NH_4-N adsorbed / Batch Mass / Contact time (Testing day 1 = 10 minutes)

In order to make sure that the adsorption was caused by MnO_2 rather then from the sand, a vessel was set up with 1400 g (0.7 L) of pure18/44 MnO_2 to test NH_4^+ adsorption (Table 8).

	MnO_2 Batches
NH_4-N adsorbed in µg	1,216
NH_4-N adsorbed per litre of media in µg L^{-1}	1,737
NH_4-N adsorbed efficiency in %	75.81
Efficiency of MnO_2 batch in comparison to control batch in %	-7.71
Average adsorption rate in µg/g/min.	0.09

Table 8: Results of ammonia adsorption trials from pure MnO_2 batches. Values are the mean of two replicates.

The results of these trials indicate that NH_4^+ is strongly adsorbed by both media. The adsorption results include the amount of NH_4^+ oxidised to NO_3^- during the batch tests (Table 7 and 8). To summarize the following evidence was collected:

Ammonia solution (10 mg L^{-1} as NH_4-N):

- 1,978 μg and 1,378 μg retained after consecutive control batches of NH_4-N added; the NH_4-N adsorbed per litre of media was 2,456 μg L^{-1} with an adsorption rate of 0.16 μg/g/min (Table 7 and Appendix C).
- 1,942 μg and 1,468 μg retained after consecutive test batches of NH_4-N added; the NH_4-N adsorbed per litre of media was 2,505 μg L^{-1} with an adsorption rate of 0.15 μg/g/min (Table 7 and Appendix C).

Ammonia solution (5 mg L^{-1} as NH_4-N):

- 696 μg and 490 μg retained after consecutive MnO_2 batches of NH_4-N added; the NH_4-N adsorbed per litre of media was 1,737 μg L^{-1} with an adsorption rate of 0.09 μg/g/min (Table 8 and Appendix C).

Pure MnO_2 batches had lower adsorption rate compared to control and test batches. However, the NH_4^+ concentration tested during the MnO_2 batches was also lower compared to the concentration used for the control and test batches. Consequently, because experiments relating concentration to adsorption were not carried out, it was not possible to establish which media had the higher NH_4^+ adsorption rate.

Nitrite adsorption:

The results of nitrite adsorption are shown in Table 9.

	Control Batches	Test Batches
NO_2-N adsorbed in µg	0	3,618
NO_2-N adsorbed per litre of media in µg L^{-1}	0	2,641
NO_2-N adsorbed efficiency in %	0	98
Efficiency of standard batch in comparison to control batch in %		98
Average adsorption rate in µg/g/min.	0	0.16

Table 9: Results of comparative nitrite adsorption trials. Values are the mean of two replicates.

NO_2^- is strongly adsorbed by MnO_2 but not adsorbed by sand. The adsorption results include the amount of NO_2^- oxidised to NO_3^- during the batch test (Table 9). The following evidence was collected:

NO_2^- solution (10 mg L^{-1} as NO_2-N):

- 278 µg and 0 µg retained after consecutive sand batches of NO_2-N added.
- 1,850 µg and 1,770 µg retained after consecutive MnO_2 batches of NO_2-N added; 0 µg released after washing with consecutive batches of standard solution.

Nitrate Adsorption:

The results of NO_3^- adsorption are shown in Table 10.

	Control Batches	Test Batches
NO_3-N adsorbed in µg	3,220	3,166
NO_3-N adsorbed per litre of media in µg L^{-1}	2,350	2,310
NO_3-N adsorbed efficiency in %	84.73	83.31
Efficiency of standard batch in comparison to control batch in %		-1.02
Average adsorption rate in µg/g/min.	0.15	0.14

Table 10: Results of comparative nitrate adsorption trials. Values are the mean of two replicates.

Both sand and MnO_2 adsorb NO_3^-. The following evidence was collected:

Nitrate solution (10 mg L^{-1} as NO_3-N):

- 1,720 µg and 1,500 µg retained after consecutive sand batches of NO_3-N added; 2,860 µg released after washing with consecutive batches of standard solution (100 ml + 100 ml standard solution added 24 hrs after the batch trials started).
- 1,766 µg and 1,400 µg retained after consecutive test batches of NO_3-N added; 2,920 µg released after washing with consecutive batches of standard solution.

Ammonia oxidation:

The results of the MnO_2 trials for the NH_4^+ oxidation to NO_3^- are shown in Table 11. NO_3^- and $[Mn_{sol}]$ were measured to prove the presence of a reaction processes.

	Control Batches	Test Batches
NO_3-N production in µg	20	118
NO_3-N production per litre of media in µg L^{-1}	15	86
NH_4-N removal efficiency in %	0.5	3
Rate of NH_4-N decrease to NO_3-N increase in µg/g/min.	6.6×10^{-6}	3.5×10^{-5}

Table 11: Results of the trials for ammonia oxidation to nitrate. Values are the mean of two replicates.

The calculation for the values showed in Table 11 was carried out as follows (see also appendix C):

1. NO_3-N production in µg* = ([NO_3-N] Time C + Time F + Time I + Time L) x total volume test solution added
2. NH_4-N removal efficiency in % = [1- (([NH_4-N] Time A + Time D) - [NO_3-N] production)/ [NH_4-N] Time A + Time D) x100]
3. Rate of NH_4-N decrease to NO_3-N increase in µg/g/min = NO_3-N production / Batch Mass / Contact time (Testing day 1 and 2 = 24 hours)

It appears from these results that NH_4^+ is only slowly converted to NO_3^- even with prolonged contact times: The NO_3-N production rate was 1.8×10^{-4} µg /g/min (Table 11) using test batches with 2,505 µg NH_4-N adsorbed per litre of media (Table 7). Initial trials with standard solution showed an output of [Mn_{sol}], measured at solubility rates of 9.2×10^{-2} µg /g/min and 6.4×10^{-2} µg /g/min after consecutive batches

* *100 ml test solution added during Time A + 100 ml test solution added during Time D = 200 ml solution added.*

(Appendix C), but this is probably related to residual fine dust still present on the surface of the MnO_2 media at the start of the trials. It is unlikely that this represents "soluble Mn" as a pH 7.03 – 7.13 the solubility of Mn is very low.

The addition of NH_4^+ increases the output of $[Mn_{sol}]$ compared with standard solution. In particular during these set of trials the initial solubility rates of $[Mn_{sol}]$ was measured at 1 x 10^{-1} µg /g/min and 1.2 x 10^{-1} µg /g/min after consecutive batches of NH_4^+ were added. This rate slowed significantly with a value of 4.3 x 10^{-4} µg /g/min after 24hours of contact time (Appendix C – Test batch with 10 mg L-1 NH_4-N).

Nevertheless from these results it is difficult to establish if the initial increase in output of manganese was caused by adsorption and/or reaction between MnO_2 and NH_4^+. Moreover given that NH_4Cl will dissociate in water as NH_4^+ and Cl^- and that

$$NH_4^+ \leftrightarrow NH_3(aq) + H^+$$

It is possible that an increased output of manganese could be generated by pH changes in the solution tested, but outlet pH was not recorded during these trials.

The $[Mn_{tot}]$ values measured during the metabolite reaction batch trials are considerably higher compared to the results obtained with the manganese solubility batch trials. A smaller volume of media was used for the manganese solubility batch trials because of the consecutive number of tests needed to be carried out with the same media. In this way by reducing the size of the batches the risk of residual solution from previous tests was thought to be reduced. Therefore it is possible that in

the case of the manganese solubility batch trials the media was washed more effectively and consequently with less manganese residual output during the test.

Nitrite oxidation:

The results of the MnO_2 trials for the NO_2^- oxidation to NO_3^- are shown in Table 12. As for the NH_4^+ oxidation trials, NO_3^- and soluble manganese were measured to prove the presence of a reaction processes.

	Control Batches	Test Batches
NO_3-N production in µg	20	3,406
NO_3-N production per litre of media in µg L^{-1}	14	2,486
NO_2-N removal efficiency in %	0.5	92
Rate of NO_2-N decrease to NO_3-N increase in µg/g/min.	6.6×10^{-6}	1.0×10^{-3}

Table 12: Results of the trials for nitrite oxidation to nitrate. Values are the mean of two replicates.

In the presence of MnO_2, NO_2^- is rapidly oxidised to NO_3^- with almost 50% conversion after 10 minutes. In particular 1,350 µg of NO_3-N was isolated after 10 minute from two consecutive MnO_2 batches to which NO_2^- was added.

Table 13 shows that the addition of NO_2^- produces a very high output of Mn and that Mn output appears to be proportional to the concentration of NO_2^- added and to the output of NO_3^-. The pH of both solutions is identical and therefore it appears as if a stoichiometric relationship is occurring. However, when looking at manganese produced, the $[Mn_{sol}]$ is far too low considering the Bartlett (1981) equation:

$$NO_2^- + MnO_2 + 2H^+ \leftrightarrow Mn^{2+} + NO_3^- + H_2O$$

For example:

10 mg L^{-1} NO_2-N = 0.01 g L^{-1} / 14 g mol^{-1} = 7.14 x 10^{-4} M

345 µg L^{-1} Mn = 345 x 10^{-6} g L^{-1} / 54.94 g mol^{-1} = 6.28 x 10^{-6} M

This implies that either the equation does not hold or that a significant amount of soluble Mn is being re-deposited as soluble Mn travels through the bed (Table 13).

Time	$[Mn_{sol}]$ µg L^{-1} $[NO_2\text{-}N]$ = 10 mg L^{-1}	$[Mn_{sol}]$ µg L^{-1} $[NO_2\text{-}N]$ = 5 mg L^{-1}
After 1st add	345 µg L-1	161 µg L-1
After 2nd add	422 µg L-1	210 µg L-1
24 hrs later	639 µg L-1	365 µg L-1

Table 13: Results for soluble manganese related to nitrite oxidation to nitrate. Values are the mean of two replicates.

3.3.4 Pilot trials of manganese performance and solubility

Standard solution:

The results of the pilot trials are shown in Table 14.

Time (hrs)	NO_2-N (mg L^{-1})	NO_3-N (mg L^{-1})	Total Mn (µg L^{-1})	Sol. Mn (µg L^{-1})	pH	Dissolved O_2 (%)
24*	4.80	0.20	2.00	1.00	7.47	80.00
48	3.40	1.60	3.50	3.70	6.02	80.00
72	2.90	2.10	6.00	4.00	5.92	79.00
96	2.50	2.30	7.60	4.50	5.97	78.00
120	2.00	2.90	8.90	5.10	5.85	81.00
144	1.70	3.30	12.10	5.70	5.81	80.00
168	1.50	3.40	15.30	6.50	5.98	80.00
192	1.40	3.60	17.80	7.50	6.04	81.00

* *Values measured before plugging pump.*

Table 14: Results of pilot trials with standard solution. Values are the means of 2 replicates.

Figure 23 shows that MnO_2 oxidised NO_2^- to NO_3^- under dynamic conditions *i.e.* where the NO_2^- solution flowed through the media under gravity. MnO_2 acted as a weak acid and initially reduced the pH solution from 7.47 to 6.02; after the first day the pH stabilised around 6 in the pilot system (Table 14). The nitrate concentration steadily increases with time, while an equivalent amount of nitrite was removed from the pilot trials. The total manganese and $[Mn_{sol}]$ increased in the pilot system, but less then expected when compared with the batch trials (Table 13). It may be that manganese reduced during the NO_2^- oxidation to NO_3^- was re-adsorbed on the surface of the solid MnO_2 and possibly re-oxidised to Mn III-Mn IV.

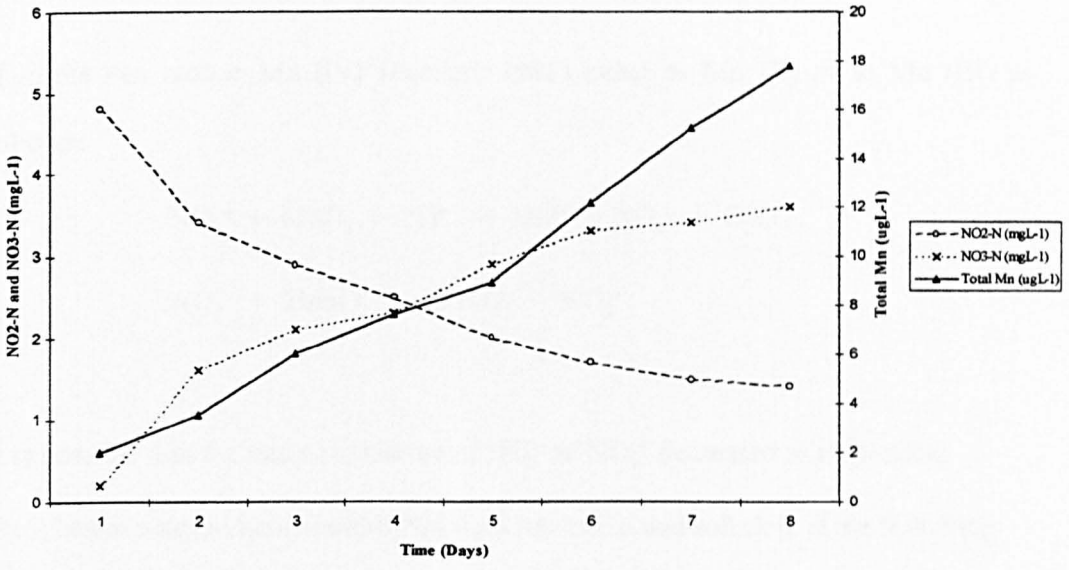


Figure 23: Nitrite oxidation and manganese solubility in pilot system.

In total 14 mg of NO_2^- as NO_2-N were oxidised to NO_3^- during day 1 compared with only 1 mg converted during day 7 (Table 14). These values were calculated as follows:

{[NO_2-N] day 1 - [NO_2-N] day 2}* Volume pilot system =

= (4.8 mg L^{-1} - 3.4 mg L^{-1}) *10 L = 14 mg

{[NO_2-N] day 7 - [NO_2-N] day 8}* Volume pilot system =

= (1.5 mg L^{-1} - 1.4 mg L^{-1}) *10 L = 1 mg

The rate of NO_2-N decrease to NO_3-N increase for the pilot filter was calculated as follows:

Day 1: (MnO_2 rate of NO_2-N decrease to NO_3-N increase) $_{SAND+\ MnO2}$ = 14000 µg / 2314 g / 1440 min. = 4.2 x 10^{-3} µg /g/min.

Day 7: (MnO_2 rate of NO_2-N decrease to NO_3-N increase) $_{SAND+\ MnO2}$ = 1000 µg / 2314 g / 1440 min. = 3.0 x 10^{-4} µg /g/min.

If nitrite can reduce Mn (IV) (Bartlett, 1981) either to Mn (II) or to Mn (III) as follows:

$$NO_2^- + MnO_2 + 2H^+ \leftrightarrow Mn^{2+} + NO_3^- + H_2O$$

$$NO_2^- + 2MnO_2 \leftrightarrow Mn_2O_3 + NO_3^-$$

It is possible that the rate of oxidation of NO_2^- to NO_3^- decreased as a chemical equilibrium was gradually established between media and solution. If the following assumptions are accepted:

- Manganese reduced during the NO_2^- oxidation to NO_3^- is adsorbed on the surface of the solid MnO_2 and re-oxidised to Mn (IV)
- The batch trials showed that both sand and MnO_2 adsorbed NO_3^- (Table 10). Consequently the increased concentration of NO_3^- in the pilot system could affect the NO_2^- adsorption on the MnO_2 and possibly reducing the rate of NO_2^- oxidation to NO_3^-.

It is possible that the different oxidation rate found between day 1 and day 7 of the pilot trials is primarily related to NO_2^- concentration, which decreased from 4.9 mg L^{-1} at day 1 to 1.4 mg L^{-1} at day 8 (Table 14). This would be the case if the $[NO_2^-]$ featured in the rate law describing the oxidation process.

Pilot trials with reconstituted soft and hard water:

The pilot trials results are presented in Tables 15 (Soft water) and 16 (Hard water).

Time (hrs)	NO_2-N (mg L^{-1})	NO_3-N (mg L^{-1})	Total Mn (µg L^{-1})	Sol. Mn (µg L^{-1})	pH	Dissolved O_2 (%)
24*	5.00	0.00	2.00	2.00	7.50	76.00
48	4.80	0.20	1.00	1.00	7.11	79.00
72	4.80	0.40	2.00	1.00	6.88	80.00
96	4.80	0.30	2.00	1.00	6.78	81.00
120	4.70	0.40	1.00	2.00	6.73	78.00
144	4.80	0.50	3.00	2.00	6.81	80.00
168	4.90	0.40	2.00	1.00	6.83	80.00
192	4.80	0.40	3.00	2.00	6.78	82.00

* *Values measured before plugging pump.*

Table 15: Results of pilot trials with reconstituted soft water. Values are the means of 2 replicates.

Under these conditions (soft and hard water), it appears that MnO_2 did not oxidise NO_2^- to NO_3^-. The failure to oxidise NO_2^- as indicated in Table 15 and 16 is in agreement with the total manganese and [Mn_{sol}] levels, which remained low throughout the entire trial. The pH values followed the same pattern observed during the pilot trials with standard solution; the pH decreased during the first day and then stabilised, probably due to surface equilibration of MnO_2.

Time (hrs)	NO_2-N (mg L^{-1})	NO_3-N (mg L^{-1})	Total Mn (µg L^{-1})	Sol. Mn (µg L^{-1})	pH	Dissolved O_2 (%)
24*	5.00	0.20	2.00	1.00	8.10	86.00
48	4.80	0.20	2.00	2.00	7.97	82.00
72	5.00	0.40	1.00	1.00	7.97	82.00
96	5.20	0.20	2.00	2.00	8.02	84.00
120	4.80	0.20	1.00	1.00	8.05	84.00
144	5.00	0.10	1.00	1.00	7.95	83.00
168	5.00	0.20	2.00	1.00	7.99	82.00
192	5.00	0.20	1.00	2.00	8.01	82.00

* *Values measured before plugging pump.*

Table 16: Results of pilot trials with reconstituted hard water. Values are the means of 2 replicates.

It appears that the constituents present in the reconstituted water inhibited the oxidation of NO_2^- to NO_3^-. This effect occurred in soft water, suggesting that the concentration of specific elements necessary to stop the NO_2^- oxidation is low. On the basis of the pilot trials results the following observations can be highlighted:

- Comparing the standard solution (made from DD water and pH adjusted with $NaHCO_3$) with reconstituted soft water, sodium and bicarbonate ions concentrations are similar, suggesting that it is unlikely these two ions are responsible for the inhibition of NO_2^- oxidation
- According to Balistrieri and Murray (1982), chloride and sulphate do not adsorb on δ- MnO_2 in the pH range of natural water

- According to Balistrieri and Murray (1982), in major ion sea water at pH = 8, 84.4% of the surface sites on δ- MnO_2 are complexed by H^+, 8.4% by Mg^{2+}, 4.6% by Ca^{2+}, 1.6% by Na^+, and 0.6% by K^+

The concentration of KCl was only 2.2 mg L^{-1} in reconstituted soft water, and hence it appears that the main ions responsible for the inhibition of NO_2^- oxidation are Ca^{2+} and Mg^{2+}. $CaSO_4$ and $MgSO_4$ was the major constituent of soft water after $NaHCO_3$ with a concentration respectively of 30.7 mg L^{-1} and 30.3 mg L^{-1}. Murray (1975) suggests that the surface charge of MnO_2 may be reversed by the exchange of monovalent surface protons for divalent cations from solution. In this context although the adsorption of $Ca^{2+}$and Mg^{2+} would enhance electrostatic attraction for an anion such as NO_2^-, an unknown surface chemistry change on MnO_2 ore would occur inhibiting the adsorption and/or oxidation of NO_2^- to NO_3^-.

As a result of the chemical investigations on the interactions between MnO_2 and nitrogen metabolites, it appears that chemical processes may not in themselves offer much potential for the application of MnO_2 as a supporting media in biofilters. Nevertheless, SEM studies suggest that MnO_2 may have a suitable surface morphology for the attachment of bacterial cells, and the possibility of favorable biological interactions merits further investigation.

Chapter 4
Hatchery effluent field trials

4.1 Introduction

The work carried out under laboratory conditions showed that Mn solubility is pH dependent and greater chemical interaction existed in distilled water solutions between MnO_2 and metabolites than the corresponding metabolites with silicate sand. The following observations were apparent:

- At an inlet pH of 5.7, $[Mn_{sol}]$ increases and appears to increase with time even after 20 minutes;
- NH_4^+ appears to enhance the MnO_2 ore solubility (increased $[Mn_{sol}]$);
- NH_4^+ is strongly adsorbed by both sand and MnO_2, but the presence of MnO_2 media appears to assist the slow conversion of NH_4^+ to NO_3^-;
- NO_2^- is strongly adsorbed, and rapidly oxidized to NO_3^-, by MnO_2 but not strongly adsorbed, or oxidized, by sand;

Dissimilatory Mn (IV) reduction occurs primarily in oxygen deficient environments (see section 2.4). Similarly, curtailment of the O_2 supply inside the MnO_2 filter bed, caused by partial clogging and channeling and/or limited diffusion process into the biofilm, could encourage microorganisms that can utilize MnO_2 ore as alternate electron acceptor. Alternatively, manganese reduction inside the filter bed could occur when bacterial activity, causes a drop in the pH and E_h values below 6.75 and 350 mV respectively, producing a spontaneous reduction of manganese (Gounot, 1994).

Furthermore, various inorganic and organic compounds, produced by bacteria metabolism can act as reducing agents for Mn (IV) (Bartlett, 1981; Lovley, 1991; Bermingham, 2001).

In contrast, autotrophic or mixotrophic bacteria may have the ability to utilize Mn (II) as a source of electrons for the immobilization of inorganic carbon into biomass (direct oxidation). Indirect microbial oxidation of Mn can alternatively occur from the bacteria production of hydrogen peroxide, free radical or oxidant species (see section 2.4).

The primary concern regarding the application of MnO_2 in an aquaculture context remains the high toxicity of manganese to fish. Direct and/or indirect microbial reduction of manganese described above would make MnO_2 ore unsuitable as bio-filter media in aquaculture applications. However, if the microbial reduction of MnO_2 was followed by chemical and /or biological re-oxidation, the process would result in constant regeneration of the interface MnO_2 ore / biofilm inside the bio-filter bed. This, in turn, will contribute to maintain a thin and active biofilm and by altering surface morphology, will increase the size of the internal surface available to bacteria. Furthermore, it is possible that MnO_2 will develop a different biological community compared with conventional bio-filters and so the competitive relation between nitrifying bacteria and heterotrophic bacteria could be modified.

To field-test the MnO_2 filter medium two pressurized filters were installed in a commercial fish farm in order to compare the metabolite removal performance of silicate sand and pure MnO_2 media in systems that are more representative of the

biological activity found in a bio-filter. The sand medium was chosen as a control to investigate MnO_2 as a supporting media for bio-filters because it is commonly used in biological filters and because silicate sand and MnO_2 appear to have comparable surface morphology and equivalent surface area available to bacteria. As a result the degree of metabolite removal between sand and MnO_2 should be mainly a function of:

- The presence of chemical interactions between the mediums and the metabolites in relation to similar water chemistry environment.
- The interactions between the microbial growth/activities and the mediums within a specific biological community established inside the filters bed.

Based on the above approach, VR and SG were excluded from the field trials because of their relatively smooth 'micro-surface' compared to the porosity found for MnO_2. In contrast, GAC was excluded because of the higher surface area available to bacteria compared to MnO_2.

However, the chemical interactions between the MnO_2 and the metabolites appeared to be negligible under the lab conditions, while the physical structure of the manganese ore seemed to provide a good "anchor" for bacteria attachment. Consequently, the degree of metabolite removal by sand and MnO_2 filters was expected to be mainly a function of the interactions between the microbial growth/activities and the media within a specific biological community established inside the filter beds.

4.1.1 RASs characteristics used for the field research

The field research was carried out in the Quoys salmon hatchery owned by Alexander Sandison & Son Ltd and based in Unst in the Shetland Islands. The hatchery produces 700,000 smolts per year of Atlantic Salmon (*Salmo salar* L.), and contributed about 40% of Shetland's smolt production.

In the beginning the hatchery was a conventional flow-through system where the water was used only once and then discharged. The Loch of Cliff was used as the water supply for intensive rearing of salmon fry, parr and pre-smolts. Historically, there were two times in the year when the hatchery experienced significant fish mortality, namely in spring amongst yolk sac fry, and in autumn amongst parr and pre-smolt. In both cases extensive gill damage was clearly visible under the microscope. An investigation into the causes of this highlighted the unsteady water chemistry of the Loch of Cliff with respect to a high concentrations of iron and manganese.

Consequently, the hatchery design was converted into a closed recirculation system in order to minimize the raw loch water consumption and improve its quality by physical filtration and ozonation treatment. The raw loch water intake was reduced to a maximum of 5% of the total water being delivered at any time to the fish tanks. As a result, the amount of make-up water used dropped from about 10,000 m^3/day in the original flow-through system to only 250 m^3/day in the RASs.

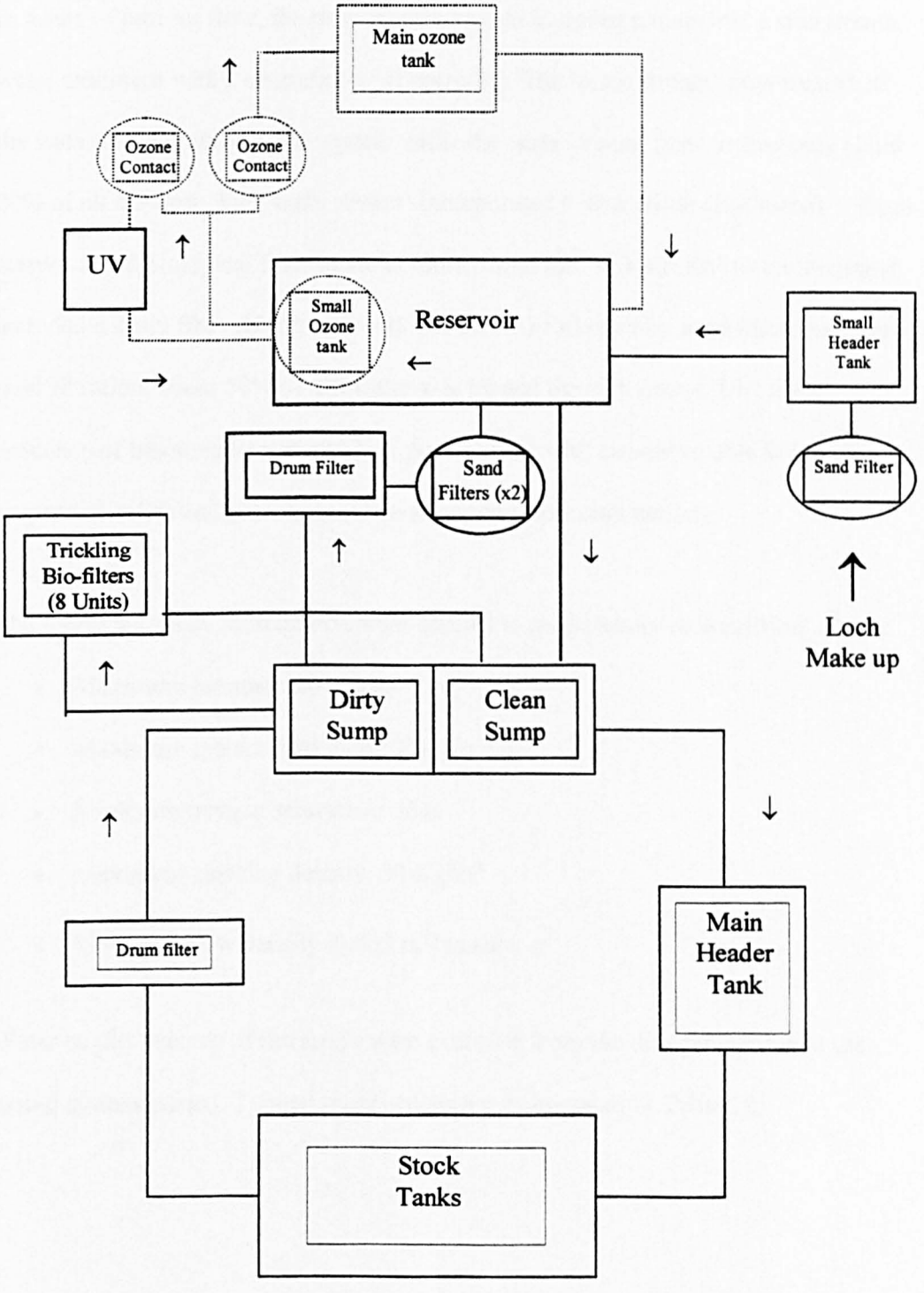
The relationships between volumes, water flows and recycle rate of the RASs are presented in Table 17. This shows that the relationship between fish rearing unit and treatment unit volume was in agreement with the RASs characteristics discussed by Rosenthal (1993), indicating that the volume of the treatment unit was 24% of the total system volume.

System volume:	
Max system volume (m^3)	1046
Fish rearing unit (m^3)	795
% Total volume	76.0
Volume treatment unit (m^3)	251
% Total volume	24.0
Degree of recycling:	
Make-up ($L\ s^{-1}$)	3
Max flow rate ($L\ s^{-1}$)	160
% Recycle = Qr/Qt (%age)	98.1
Replacement per day ($m^3\ day^{-1}$)	259.2
%age per day	24.8
System residence time (days)	4

Table 17: Quoys hatchery RASs characteristics.

The degree of recycling is expressed in both % recycle and %age per day. The former refers to % of flow exchange during each turnover, regardless of the time required for each cycle. In Table 17 98.1% recycling means that 1.9% of the flow is exchanged during each turnover. The %age per day refers to the water volume of the system exchanged per day, regardless of the number of cycles during this time period. Table

Figure 24: Quoys RASs layout.



17 indicates that 24.8 % of the total water volume (1046 m^3) is exchanged every day, which corresponds to a replacement per day of 259.2 m^3.

In terms of process flow, the recirculation system included a main and a side stream water treatment with a central sump (Figure 24). The 'main stream' flow treated all the water flowing through the system while the 'side stream' flow treated only about 20% of all the flow. The 'main stream' incorporated a drum filter (Hydrotech – 90μm screen) and 8 biological filter modular units, while the 'side stream' water treatment included a drum filter (Hydrotech – 40 μm screen) followed by sand filtration. After sand filtration, about 50% of this water was treated through ozone, UV; these sequence of treatments produced high purity water with non-detectable levels of suspended solids and pathogens (Owers, personal communication).

The following production criteria were applied to the hatchery recirculation system:

- Maximum biomass: 20 tonnes
- Maximum system feed input: 250 Kg day^{-1}
- Minimum oxygen saturation: 70%
- Maximum stocking density: 50 Kg/m^3
- Minimum flow density: 0.005 m^3/ tonne x s

Water quality records of the RASs were available from the date production in the closed system started. Typical range values are represented in Table 18.

Temperature C	4 - 18
pH	6.5 - 7.5
Oxygen (% Sat.)	60 -80
Eh (mV)	180 - 280
E.Conductivity ($\mu S\ cm^{-1}$)	380 - 400
Chloride ($mg\ L^{-1}$)	70 - 90
TSS ($mg\ L^{-1}$)	0.1 - 5
BOD ($mg\ L^{-1}$)	5 - 15
Alkalinity ($mg\ L^{-1}$ as $CaCO_3$)	30 - 50
Hardness ($mg\ L^{-1}$ as $CaCO_3$)	50 - 100
Free CO_2 ($mg\ L^{-1}$)	2 - 30
TAN ($mg\ L^{-1}$ as Nitrogen)	0.1 - 1.24
Nitrite ($mg\ L^{-1}$ as Nitrogen)	0.1 - 0.35
Nitrate ($mg\ L^{-1}$ as Nitrogen)	1 - 100
Total Iron ($\mu g\ L^{-1}$)	10 - 60
Total Manganese ($\mu g\ L^{-1}$)	20 - 40

Table 18: Characteristics of Quoys RASs water.

Water quality characteristics are closely related to feed input. The feed input ranged from a minimum of 50 Kg day^{-1} to a maximum of 250 Kg day^{-1} depending on biomass and temperature of the RASs.

4.2 Materials and methods

4.2.1 Equipment

Temperature and pH were measured with a WTW pH 320 instrument. Standardization was carried out using commercially supplied pH buffer solutions at pH 7.00 and pH 4.00, and measurements were recorded after the pH electrode was immersed in solution for approximately 5 minutes. Dissolved oxygen was measured with WTW Oxi 320 instrument. Alkalinity, hardness, ammonia and nitrite were measured immediately after sample collection with a spectrophotometer based water analysis kit (Palintest, Model 5000).

The results of comparative testing of the kit accuracy against the methods of measuring TAN (Total ammonia-nitrogen) and nitrite-nitrogen (NO_2-N) concentrations, used during the laboratory trials, are shown in Appendix B. A 'UNICAM 939' AA spectrometer equipped with a 'UNICAM GF 90' furnace was used for the quantitative analysis of total Mn. Ultra-pure nitric acid 99.99% (Fisher) was used to acidify samples (1ml HNO_3 in 50 ml sample) collected from the inlet and outlet of the filters for the quantitative analysis of total Mn. Samples were refrigerated until analysed. The material and methods used for measurement of alkalinity, hardness, BOD and TSS (Total suspended solid) are described in Appendix B (Stirling, 1985).

4.2.2 Materials

Two pressurized filters were set-up in a commercial RAS, using Lacron filters – 762-30 " (Table 19); detailed technical characteristics, filter design and configuration is showed in Appendix D.

Overall Height (mm)	811
Overall Width (mm)	762
Design working pressure (BAR)	1
Maximum working pressure (BAR)	1.5
Filtration area (m^2)	0.46
Maximum flow rate (m^3)	22.8

Table 19: Lacron pressurised filter – 762 - 30" technical characteristics.

The bottom of both filters was filled with 10 cm of 8-10 mm angular gravel. The same volume and grade size of media was used for this experiment: one filter was filled with 88 L of 16/30 silicate sand medium while the other was filled with 88 L of 16/30 MnO_2 medium. Both materials were supplied by Ferguson Wild Ltd in sealed bags (143 Kg of sand and 175 Kg of MnO_2).

Two pumps, ITT Marlow self priming centrifugal pump – type J100ec 3 phase – 0.55 kW, were used for this experiment. These pumps deliver 12 m^3/hr of water against a 10 m head, shut off head is 17 m and self priming head is 3.0 m. One pumped water from the external bottom of one fish tank (volume = 40 m^3) to a small sump (volume = 500 L); a second pumped water from this sump through the two filters and then the water was re-introduced in the outlet of the same fish tank. A valve and a flow meter

(Flow range 0-180 L/min.) was positioned at the outlet of each filters to control the flow rate (Figure 25).

Once the filter set-up was completed, a long back washing was carried out using approximately 20 m^3 (200 BVs) of system water (from main header tank); in this way residual dust from both media was removed.

Figure 25: Pressurized filters set-up in a commercial RASs. The filters were fitted at the outlet with a valve and a flow meter to control the flow rate.

4.2.3 Experimental procedures

Table 20 shows the filtration and backwash characteristics of the pressurized filters.

	Silicate sand	"MnO_2"
Filtration:		
Flow rate (m^3 hr^{-1})	2.4	2.4
Size of the bed (m^2)	0.46	0.46
Velocity (m hr^{-1})	5.2	5.2
EBCT (min.)	2.3	2.3
Depth of the bed (m)	0.2	0.2
Media size (mm)	0.5 – 1	0.5 - 1
Length of run (hrs)	24	24
Backwashing: Water backwash		
Cleaning water consumption (% of output)	4	7
Backwash flow rate (m^3 hr^{-1})	10	16
Water up flow velocity (m hr^{-1})	22	34
Filter bed fluidisation (%)	15	15

Table 20: Characteristics of the pilot filters

The flow rate was adjusted to obtain inside the filter beds a water velocity of about 5 m/hr, which is comparable to the average velocity found in typical rapid sand filters (Franklin and Pilkingston, 2001). This velocity together with a bed depth of 20 cm means that the Empty Bed Contact Time (EBCT) was maintained at over 2 minutes (Table 20).

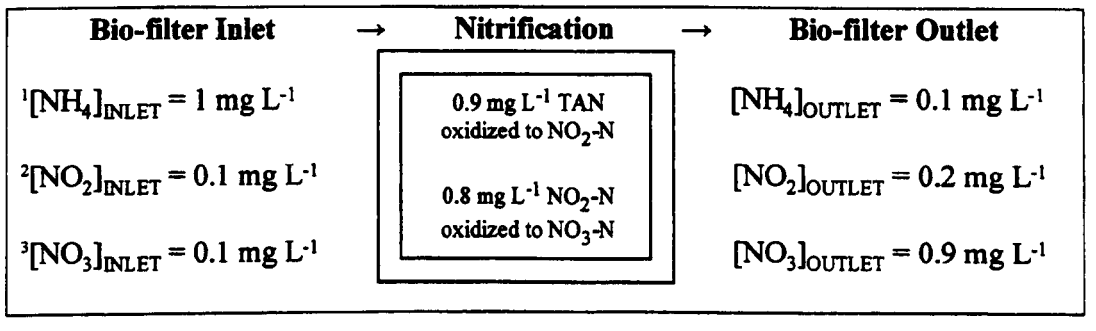
Water velocity inside the filter bed and EBCT are calculated as follows:

Velocity = Flow rate / Size of the Bed = 2.4 m^3 hr^{-1} / 0.46 m^2 = 5.2 m hr^{-1}

EBCT = Depth of the bed / Velocity = 0.2 m / 5.2 m hr^{-1} = 0.04 hr x 60 = 2.3 min.

Backwashing is an essential part of the rapid filtration process. As a filter becomes clogged, backwashing is required to remove the clogging deposits so that filtration continues in an efficient manner. Table 20 shows that backwashing of the filters was carried out every 24 hrs. Because of the lower density of sand compared with MnO_2 the backwashing flow rate for manganese medium was higher than for sand; by doing this the same level of bed fluidisation was maintained in both filters. In addition twice a week, prior to backwashing, the filter beds were carefully stirred by hand for 2 minutes to ensure that the media were not 'sticky' or compacted in certain areas.

Nitrifying systems oxidise ammonia via nitrite to nitrate, but where the oxidation of ammonia is incomplete, nitrite accumulates in RASs. Thus, high removal rates of ammonia by the filter do not necessary imply an overall improvement of system performance (Figure 26). Filter performance was evaluated by monitoring the change in TAN and NO_2-N as recycled water passed through each filter, assuming that TAN and NO_2-N were converted to nitrate.



[1] *$[NH_4]_{INLET}$ = Concentration of NH_4^+ as mg of TAN at the inlet of the filters.*
[2] *$[NO_2]_{INLET}$ = Concentration of NO_2^- as mg of Nitrogen at the inlet of the filters.*
[3] *$[NO_3]_{INLET}$ = Concentration of NO_3^- as mg of Nitrogen at the inlet of the filters.*

Figure 26: Example bio-filter nitrogen mass balance showing incomplete ammonia oxidation to nitrate with accumulation of nitrite. In this example the absolute nitrite removal ($[NO_2]_{INLET} - [NO_2]_{OUTLET}$) is negative giving a nitrite output of 0.1 mg L^{-1} NO_2-N as a result of an incomplete ammonia oxidation of 0.9 mg L^{-1} TAN to nitrate.

The two pilot filters were sampled for a period of 24 weeks, organized as follows:

1) During the biological start-up, time during which the ammonia and nitrite removal started and gradually increased, water samples were collected every second day, 24 hours after filter backwashing.

2) When the biological start up was completed, *i.e.* when the removal efficiency of ammonia and nitrite reached a plateau (stable levels which were not exceeded on successive days); samples were collected twice a week, 2 and 24 hrs after backwashing the filters. Additionally, during a limited period of time, samples were also collected 4 and 6 hrs after backwashing the filters. These tests were carried out to investigate the effect of backwashing the filters on metabolite removal performance.

3) Finally in order to examine the effect of contact time, the depth of the bed was reduced to half (from week 20 to week 24), bringing the media volume from 88 to 44 L. The flow rate was not changed and therefore the same water velocity was maintained inside the filter bed. As a result the contact time was reduced to just over 1 minute. As in the other trials, samples were collected twice a week, 2 and 24 hrs after backwashing the filter.

The filter trials started when the RAS production cycle was at its beginning and ended in the middle of the same production cycle (week 24). During this period the feed input in the system fluctuated from a minimum of 50 kg day^{-1} to a maximum 250 kg day^{-1}. As a result various water quality parameters such as metabolite, TSS and BOD fluctuated during the trial period. Finally the water temperature of the recirculation

unit was directly linked to the ambient temperature. Because the trial started in summer and ended in winter, a relatively wide temperature range occurred during this investigation. Consequently, in addition to the change in metabolites and oxygen as recycled water passed through each filter, samples were regularly collected from the inlet (small sump feeding the filters) of the filters in order to monitor the following water parameters:

- Temperature and pH
- Alkalinity (mg L^{-1} as $CaCO_3$)
- TSS (in mg L^{-1})
- BOD_5 (mg L^{-1}as O_2)

4.3 Results

4.3.1 Biological start-up period

The data in Table 21 shows the range of ambient conditions during the filter start-up period. Measurements were carried out over a 4-weeks period. The collected data are listed in Table 22.

	Sample No.	Range	Average
Temperature C	14	13.00 - 16.00	14.74
pH	14	7.15 - 7.39	7.27*
Alkalinity (mg L^{-1} as $CaCO_3$)	8	26.00 - 50.00	38.00
Hardness (mg L^{-1} as $CaCO_3$)	8	80.00-120.00	100.00
Dissolved Oxygen (mg L^{-1})	14	9.10 - 10.20	9.80
TSS (mg L^{-1})	8	0.65 - 1.00	0.90
BOD_5 (mg L^{-1}as O_2)	8	8.7 - 10.8	10.20
TAN (mg L^{-1}as Nitrogen)	14	0.08 - 0.42	0.19
Nitrite (mg L^{-1} as Nitrogen)	14	0.02 - 0.10	0.05

** Arithmetic average of pH values not [H^+]*

Table 21: Range of ambient conditions during filters biological start-up period.

Table 22 shows that the two filters behaved similarly during the biological start up period. At an average inlet ammonia concentration $[NH_4]_{INLET}$ of 0.19 mg L^{-1} TAN and average temperature of 14.7 °C, the biological start-up, with respect to ammonia oxidation, was almost completed in three weeks from filter set up (sample 9 – week 3). At that time in both filters, the ammonia removal efficiency reached more than 80% but there was still a net production of nitrite ($[NO_2]_{INLET} - [NO_2]_{OUTLET} < 0$),

which stopped at the end of week 4 (sample 14), suggesting the beginning of the biological start up with respect to nitrite removal.

	TAN in mg L^{-1}			$[NO_2\text{-}N]$ in mg L^{-1}				
	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	pH	Temp.(°C)
Sample 1 - (Week 1)	0.08	0.10	0.07	0.02	0.02	0.03	7.30	13.00
Sample 2 - (Week 1)	0.09	0.07	0.06	0.02	0.03	0.03	7.23	14.00
Sample 3 - (Week 2)	0.10	0.07	0.07	0.02	0.03	0.04	7.35	14.80
Sample 4 - (Week 2)	0.10	0.04	0.06	0.02	0.05	0.04	7.33	14.80
Sample 5 - (Week 3)	0.12	0.06	0.06	0.02	0.04	0.03	7.29	14.30
Sample 6 - (Week 3)	0.12	0.05	0.04	0.02	0.04	0.04	7.39	14.80
Sample 7 - (Week 3)	0.13	0.04	0.02	0.06	0.10	0.08	7.31	14.30
Sample 8 - (Week 3)	0.26	0.05	0.04	0.08	0.14	0.13	7.27	14.20
Sample 9 - (Week 3)	0.19	0.03	0.02	0.06	0.09	0.10	7.18	15.20
Sample 10 - (Week 4)	0.20	0.02	0.02	0.06	0.07	0.08	7.15	15.60
Sample 11- (Week 4)	0.24	0.03	0.01	0.05	0.08	0.08	7.28	14.90
Sample 12 - (Week 4)	0.22	0.03	0.02	0.07	0.08	0.06	7.38	15.20
Sample 13 - (Week 4)	0.33	0.02	0.02	0.08	0.09	0.08	7.22	15.30
Sample 14 - (Week 4)	0.42	0.04	0.06	0.10	0.09	0.08	7.15	16.00
Average	0.19	0.05	0.04	0.05	0.07	0.06	7.27	14.74
s	0.10	0.02	0.02	0.03	0.03	0.03	0.08	0.75
Removal Efficiency (in %)		75.00	78.08		-41.59	-33.63		
Removal Rate (in g/m3/day)		87.20	90.78		-12.39	-10.02		

Table 22: Pilot filters observations carried out during the biological start-up period.

The following formulas were used to calculate removal efficiency and removal rate (Muir, 1982): where

Ci and *Cf* indicate respectively inlet and outlet ammonia and nitrite concentration from bio-filter units.

Removal efficiency (in %) = $[1 - (Cf / Ci)] * 100$

Example 1: Ammonia Removal Efficiency Sample 5 (Sand filter) = [1- (0.06 mg L^{-1} / 0.12 mg L^{-1})] * 100 = 50.00 %

Removal rate (in $g/m^3/day$) = {[(*Ci* - *Cf*) * flow rate] / Unit Volume Media

Example 2: Ammonia Removal Rate Sample 5 (Sand filter) = {[(0.12 mg L^{-1} – 0.06 mg L^{-1}) * 57.60 m^3 day^{-1}] / 0.088 m^3 = 39.27 $g/m^3/day$

4.3.2 Observations carried out after biological start up was completed

The data presented in Table 23 shows the range of ambient conditions at filter start up. Measurements were carried out over a13-week-period.

	Sample No.	Range	Average
Temperature (^{o}C)	43	9.40 - 17.00	14.40
pH	43	6.80 - 7.40	6.96
Alkalinity (mg L^{-1} as $CaCO_3$)	14	26.00 - 50.00	38.00
Dissolved Oxygen (mg L^{-1})	43	8.40 - 11.20	9.73
TSS (mg L^{-1})	14	1.00 - 2.00	1.80
BOD_5 (mg L^{-1}as O_2)	14	10.40 - 15.20	13.50
TAN (mg L^{-1}as Nitrogen)	43	0.20 - 1.66	0.98
Nitrite (mg L^{-1} as Nitrogen)	43	0.07 - 0.26	0.18

Table 23: Range of ambient conditions after filters biological start-up was completed.

Backwashing the filter decreases the accumulation of solids and contributes to controlling the growth of heterotrophic bacteria, by increasing substrate (ammonia, nitrite and particularly oxygen) availability for the desired nitrifying autotrophic bacteria. However, filter backwashing can also reduce the nitrifying autotrophic bacteria biomass by partially removing bacterial cell and bacteria flocs attached to the surface of the media and/ or to solid particles trapped in the filter bed (Golz *et al.*, 1999). In this case, bio-filter performance will be almost certainly affected because autotrophic organism doubling time is more than an order of magnitude slower than that of aerobic heterotrophs. Watson (1971) and Bock *et al.* (1989) suggest a doubling time for nitrifying bacteria of 7 – 8 h under ideal conditions, while Shilo and Rimon (1982) measured a doubling time of 26 h for ammonia oxidizer and 60 h for nitrite

oxidizer nitrifiers present in fishponds. Thus, in order to study the effect of filter backwashing on filter performance with respect to bacterial population, additional measurements were carried out 2, 4, 6 and 24 hours after backwashing to compare ammonia and nitrite removal efficiency and removal rate between the sand and MnO_2 pilot filters. The collected data are listed in Table 24, 25 and 27.

Measurements carried out 24 hours after filter backwashing

	TAN in mg L^{-1}			[NO_2-N] in mg L^{-1}			[O2] in mg L^{-1}				
	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	pH	Temp.(°C)
Sample 1- (Week 5)	0.42	0.04	0.06	0.10	0.09	0.08	9.30	6.50	6.60	7.15	16.00
Sample 2- (Week 5)	0.32	0.02	0.02	0.08	0.06	0.04	9.00	6.40	6.40	7.19	16.50
Sample 3 - (Week 5)	0.20	0.05	0.01	0.07	0.02	0.01	9.30	6.80	6.80	7.22	16.90
Sample 4- (Week 6)	0.33	0.03	0.01	0.08	0.05	0.03	9.30	6.70	6.70	7.36	17.00
Sample 5- (Week 6)	0.42	0.04	0.04	0.10	0.05	0.03	9.10	6.20	6.10	7.40	16.50
Sample 6- (Week 7)	0.61	0.04	0.04	0.12	0.07	0.06	10.80	6.50	6.60	7.00	16.10
Sample 7 - (Week 8)	1.00	0.13	0.10	0.17	0.22	0.11	8.70	3.20	3.00	7.00	15.90
Sample 8- (Week 8)	1.29	0.12	0.04	0.19	0.27	0.10	10.20	3.94	3.43	6.93	16.00
Sample 9- (Week 9)	1.32	0.11	0.09	0.18	0.24	0.09	9.20	2.40	2.20	6.93	16.40
Sample 10- (Week 9)	1.06	0.06	0.05	0.17	0.12	0.04	10.60	3.84	3.85	7.07	16.10
Sample 11 - (Week 10)	1.32	0.06	0.04	0.17	0.13	0.03	10.40	3.85	3.58	6.96	16.10
Sample 12- (Week 10)	1.42	0.07	0.05	0.19	0.14	0.03	9.57	2.42	2.17	6.88	16.30
Sample 13- (Week 11)	1.22	0.09	0.05	0.19	0.14	0.02	10.28	3.81	3.48	6.90	15.60
Sample 14- (Week 11)	1.54	0.19	0.09	0.18	0.28	0.12	8.87	2.16	1.73	6.81	14.90
Sample 15 - (Week 13)	0.83	0.10	0.03	0.18	0.08	0.01	9.97	5.48	5.22	7.05	13.70
Sample 16- (Week 13)	0.83	0.07	0.03	0.19	0.06	0.01	9.43	5.27	5.04	6.92	13.90
Sample 17- (Week 13)	0.87	0.06	0.03	0.21	0.08	0.01	10.09	5.70	5.42	6.91	14.00
Sample 18- (Week 14)	0.61	0.03	0.02	0.16	0.05	0.00	9.93	6.08	5.80	6.96	13.80
Sample 19 - (Week 14)	0.48	0.05	0.03	0.14	0.04	0.01	9.38	6.15	5.84	7.18	13.30
Sample 20- (Week 15)	1.15	0.06	0.04	0.20	0.12	0.02	8.81	3.84	3.40	6.86	13.10
Sample 21- (Week 16)	0.91	0.09	0.05	0.21	0.12	0.02	9.50	4.27	3.81	6.87	12.70
Sample 22- (Week 18)	1.25	0.10	0.08	0.24	0.16	0.03	8.94	3.05	2.90	6.77	12.30
Sample 23 - (Week 18)	1.00	0.11	0.06	0.16	0.12	0.02	9.80	5.11	4.60	6.90	11.00
Sample 24- (Week 19)	0.96	0.09	0.04	0.17	0.15	0.03	9.66	4.35	3.73	6.94	10.60
Sample 25- (Week 19)	1.00	0.20	0.09	0.15	0.16	0.03	10.15	5.41	4.93	6.80	9.40
Sample 26- (Week 19)	0.91	0.06	0.03	0.15	0.11	0.02	9.28	4.88	4.81	6.93	11.00
Average	0.90	0.08	0.05	0.16	0.12	0.04	9.60	4.78	4.54	7.00	14.43
s	0.37	0.05	0.03	0.04	0.07	0.03	0.58	1.45	1.54	0.16	2.20
Removal Efficiency (in %)		91.10	94.76		24.86	76.04		50.19	52.66		
Removal Rate (in g/m3/day)		510.50	530.97		24.85	76.02		3016.05	3164.63		

Table 24: Pilot filter observations carried out 24 hours after filter backwashing.

From the measurements carried out 24 hrs after filter backwashing, the average ammonia removal efficiency was comparable in the two pilot filters; the sand filter had an average efficiency of 91.10% compared to 94.76% of MnO_2. However, the

MnO_2 pilot filter showed a considerably higher nitrite removal efficiency; at an average 76.04% compared to 24.86% for the sand filter (Table 24).

Figure 27 compares the relationship between $[NH_4]_{INLET}$ and ammonia removal ($[NH_4]_{INLET} - [NH_4]_{OUTLET}$) for the two pilot filters. The correlation coefficient R^2 between these parameters was high ($R^2_{SAND} = 0.99$; $R^2_{MnO_2} = 0.9969$) for both filters. As expected, increased removal was found with increasing inlet concentration, which indicates that the filters media were operating within their maximum loading. As the $[NH_4]_{INLET}$ did not exceed 1.54 mg L^{-1} TAN (Figure 27), the maximum removal rate must occur at higher values (van Rijn and Rivera, 1990). However, the results shown in Figure 27 suggest that the MnO_2 medium is more efficient at removing ammonia as the $[NH_4]_{INLET}$ increases; the maximum differential observed between the two media was 0.1 mg L^{-1} TAN at inlet levels of 1.54mg L^{-1} TAN (sample 14 – week 11 in Table 24 and Figure 27). The statistical significance of this observation was not tested since the R^2 were very high for both filters.

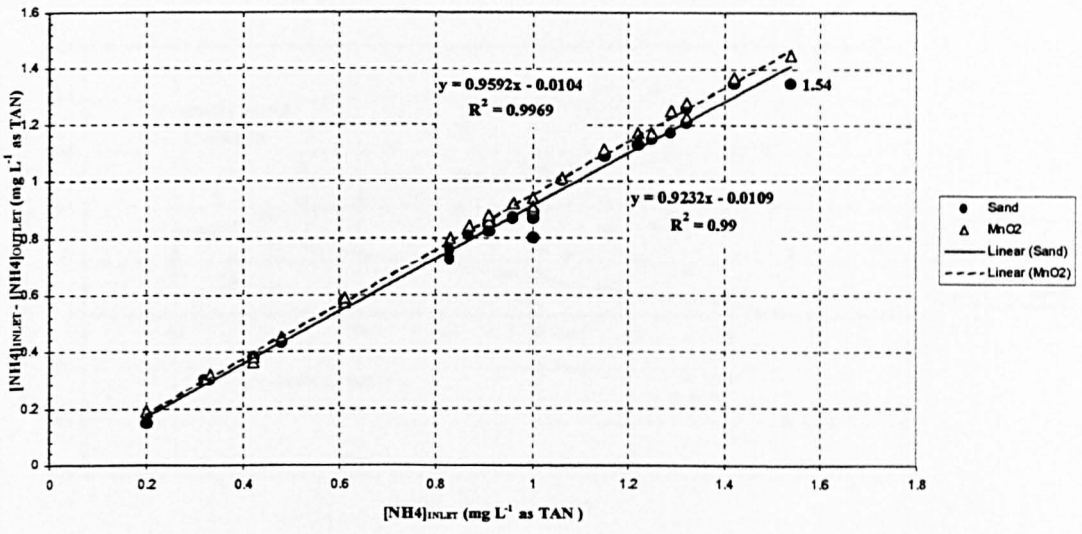


Figure 27: Observations from pilot filters carried out after biological start-up at two minute EBCT and 24 hours after backwashing. Ammonia removal ($[NH_4]_{INLET} - [NH_4]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

The average $[NH_4]_{INLET}$ measured for the pilot filters was about one order of magnitude greater than the average inlet nitrite concentration $[NO_2]_{INLET}$ (Table 24). Since the average ammonia removal efficiency was over 90% for both filters, it can be assumed that the bulk of the nitrite produced and removed inside the filter bed was the result of the oxidation of ammonia to nitrate (Figure 26). As the nitrification rate depends strongly on substrate concentrations (Srna and Baggaley, 1975; Rittmann and McCarty, 1980; Drtil *et al.*, 1993), the comparative removal of nitrite by the two pilot filters was determined by studying the relationship between $[NH_4]_{INLET}$ and absolute nitrite removal ($[NO_2]_{INLET} - [NO_2]_{OUTLET}$) (Figure 28).

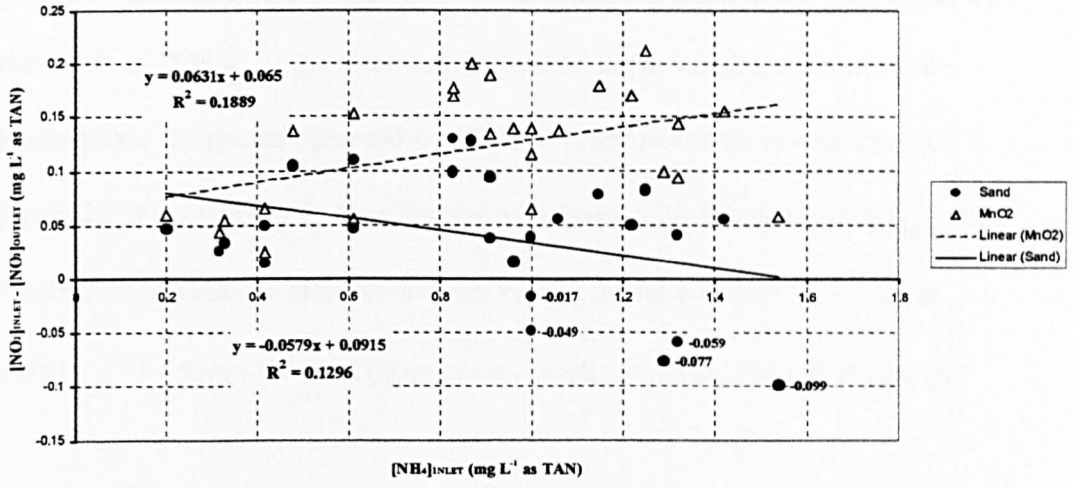


Figure 28: Observations from pilot filters carried out after biological start-up at two minute EBCT and 24 hours after backwashing. Absolute nitrite removal ($[NO_2]_{INLET} - ([NO_2]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

Figure 28 compares the relation between $[NH_4]_{INLET}$ and absolute nitrite removal for the two pilot filters. The correlation coefficient R^2 between these parameters was low for both filters ($R^2_{SAND} = 0.1296$; $R^2_{MnO_2} = 0.1889$). However in general, increased nitrite removal was found with increasing $[NH_4]_{INLET}$ for the MnO_2 filter, while decreased ($[NO_2]_{INLET} - [NO_2]_{OUTLET}$) was found with increasing $[NH_4]_{INLET}$ for the sand filter. On a few occasions, when the $[NH_4]_{INLET}$ was ≥ 1.0 mg L^{-1} TAN, the sand pilot filter appeared to have a net output of nitrite observed (Figure 28).

The nitrification process carried out by autotrophic bacteria to convert ammonia to nitrate requires oxygen. Consequently, measurements of oxygen removal ($[O_2]_{INLET} - [O_2]_{OUTLET}$) for both pilot filters were taken during the13-week period. Figure 29 compares the relation between $[NH_4]_{INLET}$ and oxygen up-take for the two filters. The

correlation coefficient R^2 between these parameters was high (R^2_{SAND} = 0.9077; $R^2_{MnO_2}$ = 0.9296) for both filters (Figure 29). As expected, increased uptake was found with increased of $[NH_4]_{INLET}$, *i.e.* more oxygen is used to convert ammonia to nitrate. Furthermore, the results observed for $[O_2]$ levels reinforces the results observed in Figure 27, the difference in O_2 uptake also increases with increasing $[NH_4]_{INLET}$; the maximum differential observed between the two media was 0.43 mg L^{-1} O_2 at $[NH_4]_{INLET}$ of 1.54mg L^{-1} TAN (sample 14 – week 11 in Table 24 and Figure 29).

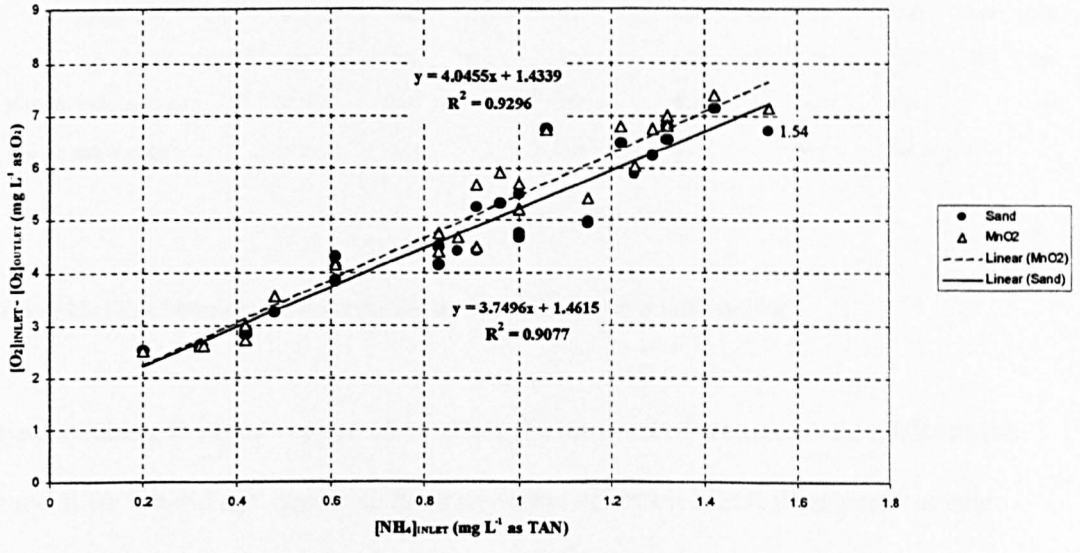


Figure 29: Observations from pilot filters carried out after biological start-up at two minute EBCT and 24 hours after backwashing. Oxygen removal ($[O_2]_{INLET} - ([O_2]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

Measurements carried out 2 hours after filter backwashing

	TAN in mg L^{-1}			[NO_2-N] in mg L^{-1}			[O2] in mg L^{-1}				
	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO2	Inlet	Outlet Sand	Outlet MnO_2	pH	Temp.(°C)
Sample 1- (Week 8)	1.25	0.26	0.06	0.18	0.26	0.11	10.00	5.20	4.03	6.93	16.00
Sample 2 - (Week 9)	1.32	0.25	0.05	0.19	0.34	0.13	9.40	4.10	2.90	6.93	16.40
Sample 3 - (Week 9)	1.32	0.25	0.04	0.19	0.34	0.11	9.20	3.50	2.60	7.07	16.10
Sample 4 - (Week 9)	1.66	0.37	0.09	0.26	0.41	0.16	10.30	4.40	2.80	6.96	16.10
Sample 5 - (Week 10)	1.35	0.26	0.06	0.19	0.25	0.07	10.30	4.70	3.60	6.88	16.30
Sample 6 - (Week 10)	1.42	0.28	0.09	0.19	0.24	0.09	11.20	5.05	4.05	6.90	15.60
Sample 7 - (Week 11)	1.22	0.25	0.06	0.21	0.22	0.06	10.45	5.22	4.32	6.81	14.90
Sample 8 - (Week 11)	1.22	0.25	0.06	0.22	0.24	0.06	10.60	5.05	4.21	6.81	14.90
Sample 9 - (Week 13)	0.60	0.11	0.01	0.18	0.11	0.01	9.74	5.99	5.35	7.05	13.70
Sample10 - (Week 13)	0.71	0.12	0.03	0.17	0.10	0.01	10.05	6.58	6.03	6.92	13.90
Sample 11 - (Week 13)	0.66	0.11	0.02	0.21	0.10	0.01	10.30	7.07	6.50	6.91	14.00
Sample 12 - (Week 14)	0.55	0.09	0.03	0.14	0.08	0.01	9.78	6.71	6.22	6.96	13.80
Sample 13 - (Week 14)	0.87	0.15	0.04	0.16	0.10	0.02	9.27	5.37	4.63	7.18	13.30
Sample 14 - (Week 15)	0.96	0.21	0.04	0.21	0.17	0.03	9.22	5.51	4.80		
Sample 15 - (Week 15)	1.15	0.31	0.06	0.19	0.21	0.04	8.40	4.43	3.43	6.86	13.10
Sample 16 - (Week 16)	0.96	0.13	0.03	0.19	0.18	0.04	9.35	5.35	4.20	6.87	12.70
Sample 17 - (Week 18)	0.91	0.19	0.09	0.18	0.17	0.05	9.55	5.27	4.85	6.77	12.30
Sample 17 - (Week 18)	0.94	0.19	0.05	0.14	0.16	0.04	10.40	6.42	5.42	6.90	11.00
Average	1.06	0.21	0.05	0.19	0.20	0.06	9.86	5.33	4.44	6.92	14.36
s	0.31	0.08	0.02	0.03	0.09	0.05	0.67	0.95	1.16	0.10	1.60
Removal Efficiency (in %)		80.18	95.23		-8.07	69.08		45.96	54.97		
Removal Rate (g m^3 day^{-1})		531.83	631.65		-9.57	81.91		2837.91	3393.74		

Table 25: Pilot filters observations carried out 2 hours after filter backwashing.

Backwashing the filters appeared to affect the removal of ammonia and nitrite in the sand filter but did not appear to have any great affect on MnO_2 filter performance. From measurements taken 2 hours after backwashing, differences between the two pilot filters with respect to removal efficiency were the following (Table 25):

- The sand filter had an average ammonia removal efficiency of 80.18% compared to 95.23% for MnO_2.
- The MnO_2 filter had an average nitrite removal efficiency of 69.08% compared to – 8.07% for sand.

Figure 28 compares the post-backwash relationship between $[NH_4]_{INLET}$ and ammonia removal for the two pilot filters. The correlation coefficient R^2 between these parameters was high ($R^2_{SAND} = 0.9872$; $R^2_{MnO_2} = 0.9971$) for both filters and increased removal was found with increasing $[NH_4]_{INLET}$. In addition, the difference in removal between that of the sand and that of the MnO_2 pilot filters increases with the increasing $[NH_4]_{INLET}$; the maximum differential observed between the two media was 0.28 mg L^{-1} TAN at $[NH_4]_{INLET}$ of 1.66 mg L^{-1} TAN (sample 4 – week 9 in Table 25 and Figure 30).

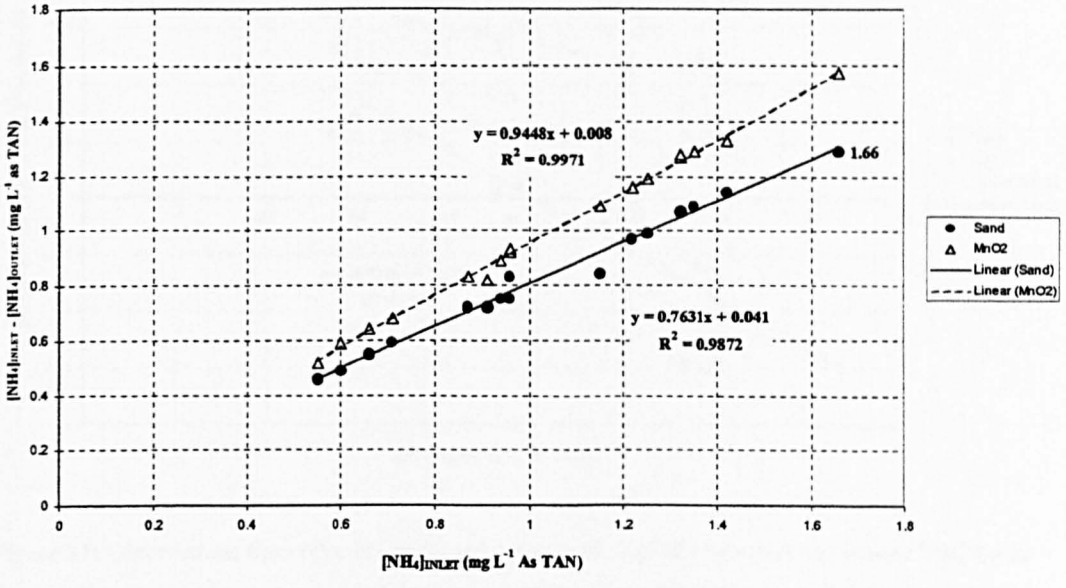


Figure 30: Observations from pilot filters carried out after biological start-up at two minute EBCT and 2 hours after backwashing. Ammonia removal ($[NH_4]_{INLET} - [NH_4]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

Figure 31 compares the relation between $[NH_4]_{INLET}$ and absolute nitrite removal ($[NO_2]_{INLET} - [NO_2]_{OUTLET}$) for the two pilot filters 2 hours after backwashing. The correlation coefficient R^2 between these parameters was low for the MnO_2 filter ($R^2_{MnO_2} = 0.3538$) but it was high in the case of the sand filter ($R^2_{SAND} = 0.773$).

However, in general decreased NO_2 removal was found with increasing of $[NH_4]_{INLET}$ for both filters at this stage of the trial. Continual increases in nitrite concentration was observed for the sand filter, when the $[NH_4]_{INLET}$ was ≥ 1.0 mg L^{-1} TAN; the maximum nitrite output from the sand filter was 0.15 mg L^{-1} NO_2-N when $[NH_4]_{INLET}$ was 1.32 mg L^{-1} as TAN. In comparison, removal of 0.10 mg L^{-1} NO_2-N from the MnO_2 filter was found (sample 4 – week 9 in Table 25 and Figure 31).

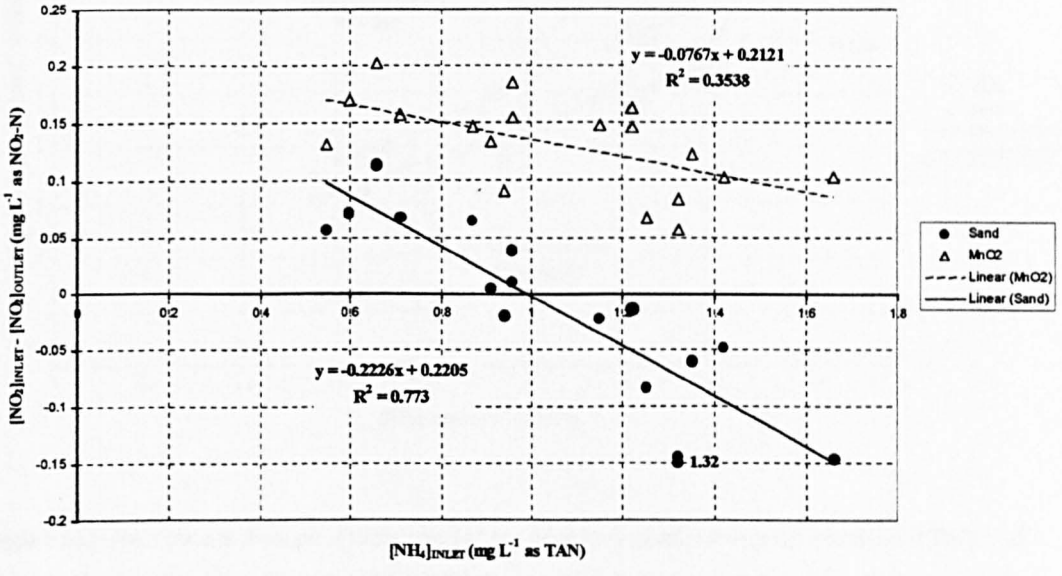


Figure 31: Observations from pilot filters carried out after biological start-up at two minute EBCT and 2 hours after backwashing. Absolute nitrite removal ($[NO_2]_{INLET} - [NO_2]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

Figure 32 compares the relation between $[NH_4]_{INLET}$ and O_2 uptake for the two filters measured 2 hours after backwashing. The correlation coefficient between these parameters was high ($R^2_{SAND} = 0.8477$; $R^2_{MnO_2} = 0.92$) for both filters (Figure 32). As expected, increased uptake was found with increasing $[NH_4]_{INLET}$. Again the difference in removal between sand and MnO_2 filters increased with the increasing

$[NH_4]_{INLET}$ (Figure 30). Similarly, the difference in O_2 uptake increased with the increasing $[NH_4]_{INLET}$; the maximum differential observed between the two media was 1.60 mg L^{-1} O_2 at $[NH_4]_{INLET}$ of 1.66 mg L^{-1} TAN (sample 4 – week 9 in Table 25 and Figure 32).

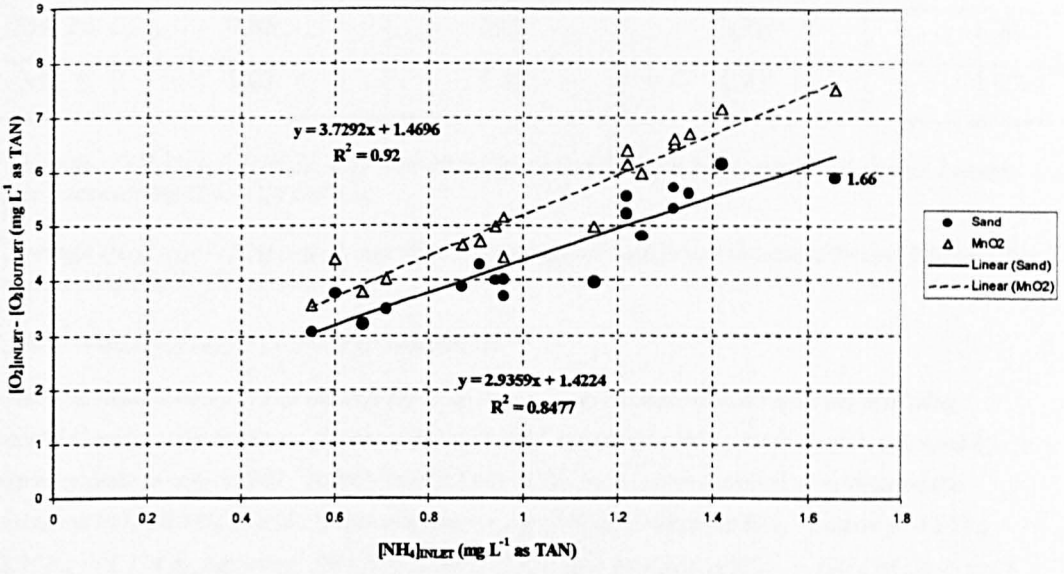


Figure 32: Observations from pilot filters carried out after biological start-up at two minute EBCT and 2 hours after backwashing. Oxygen removal ($[O_2]_{INLET} - [O_2]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

From the measurements carried out 24 and 2 hours after backwashing both filters over a 13-week period, increased ammonia removal and O_2 uptake was found for increasing $[NH_4]_{INLET}$, suggesting that the $[O_2]_{INLET}$ was used by nitrifiers to oxidize ammonia to nitrate. However, this O_2 uptake was found to exceed the theoretical oxygen requirements for ammonia removal for both pilot filters (Table 23). However, the difference between theoretical (bacterial O_2 usage) and observed uptake decreased after backwashing the filters (Table 26).

	Observed $[NH_4]_{INLET}$ - $[NH_4]_{OUTLET}$ (as mg L^{-1} TAN)[A]	Observed $[O_2]_{INLET}$ - $[O_2]_{OUTLET}$ (as mg L^{-1} O_2)[B]	Theoretical $[O_2]_{INLET}$ - $[O_2]_{OUTLET}$ (as mg L^{-1} O_2)[C]	$[O_2]_{INLET}$ - $[O_2]_{OUTLET}$ (Observed - Theoretical)
Sand 24	0.82	4.82	3.54	1.28
Sand 2	0.85	4.53	3.62	0.91
Mn 24	0.85	5.06	3.76	1.30
Mn 2	1.01	5.42	4.45	0.97

[A] *Average ($[NH_4]_{INLET}$ – $[NH_4]_{OUTLET}$) over 13 weeks-period for both filters measured 24 and 2 hours after backwashing (Table 24 and 25).*

[B] *Average ($[O_2]_{INLET}$ – $[O_2]_{OUTLET}$) over 13 weeks-period for both filters measured 24 and 2 hours after backwashing (Table 24 and 25).*

[C] *Theoretical ($[O_2]_{INLET}$ – $[O_2]_{OUTLET}$) calculation:*

From the stoichiometry of Eqs (8) and (9) in section 1.3.1 is possible to determine the nitrifying bacterial oxygen requirements: for every gram of NH_4^+ oxidized to NO_3^-, 3.158 g of O_2 are used by Nitrosomonas to convert NH_4^+ to NO_2^- and 1.114 g of O_2 are used by Nitrobacter to convert the produced NO_2^- to NO_3^-. Total O_2 consumption for 1 g of NH_4^+ oxidized to NO_3^- is equal to 4.273 g (3.158 g + 1.114 g). Moreover, for every gram of $[NO_2]_{INLET}$ oxidized to NO_3^-, 0.350 g of O_2 is used by Nitrobacter to convert NO_2^- to NO_3^-.

The following method was used to calculate the theoretical ($[O_2]_{INLET}$ – $[O_2]_{OUTLET}$):

(($[NH_4]_{INLET}$ – $[NH_4]_{OUTLET}$) x 4.273) + (($[NO_2]_{INLET}$ - $[NO_2]_{OUTLET}$) x 1.114)

Table 26: Comparison between theoretical and observed ($[O_2]_{INLET}$ – $[O_2]_{OUTLET}$) for MnO_2 and sand pilot filters.

Measurements carried out 4 and 6 hours after filter back washing

From measurements made 2 hours afterwards, backwashing the filters appeared to seriously affect the removal efficiency of ammonia and nitrite in the case of the sand filter. Considering that Watson (1971) and Bock *et al.* (1989) suggest a doubling time for nitrifying bacteria of 7 – 8 h under ideal condition, samples were taken at 4 and 6

hours after backwashing in week 11 of the trial and evaluated for NH_4^+, NO_2^- and O_2 to examine the changes in filter performance caused by backwashing.

	TAN in mg L^{-1}			[NO2-N] in mg L^{-1}			[O2] in mg L^{-1}		
	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2
Sample 1- (Week 11)	1.42	0.23	0.05	0.19	0.25	0.06	10.80	4.56	3.62
Sample 2- (Week 11)	1.22	0.21	0.04	0.21	0.22	0.05	9.70	3.96	3.07
Sample 3 - (Week 11)	1.54	0.21	0.06	0.21	0.21	0.05	10.30	4.40	3.70
Average	1.39	0.22	0.05	0.20	0.23	0.06	10.27	4.31	3.46
Removal Efficiency (in %)		84.45	96.41		-14.19	75.15		58.05	66.27
Removal Rate (g m^3 day^{-1})		832.70	926.61		-4.38	95.17		3693.91	4132.17

Table 27: Observations from the biologically active pilot filters carried out 4 hours after filter backwashing.

	TAN in mg L^{-1}			[NO2-N] in mg L^{-1}			[O2] in mg L^{-1}		
	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2
Sample 1- (Week 11)	1.42	0.14	0.05	0.19	0.17	0.04	10.80	4.30	3.40
Sample 2- (Week 11)	1.22	0.13	0.03	0.22	0.21	0.04	8.95	3.50	2.80
Sample 3- (Week 11)	1.54	0.14	0.06	0.26	0.20	0.05	11.20	4.96	4.20
Average	1.39	0.14	0.05	0.22	0.19	0.04	10.32	4.25	3.47
Removal Efficiency (in %)		90.19	96.65		14.80	78.25		58.77	66.40
Removal Rate (g m^3 day^{-1})		876.52	926.61		38.82	133.98		3906.78	4382.61

Table 28: Observations from the biologically active pilot filters carried out 6 hours after filter backwashing.

The data in Table 27 and 28 suggests that the sand filter showed improved ammonia and nitrite removal efficiency between 4 and 6 hours after back washing, while the MnO_2 filter did not show time dependant removal efficiency over this time period. The data in Table 29 summarises the observations on the effects of backwashing in the 24 hours life cycle of the filters during the 13 week-period of the trial.

Medium (hr)	N. Sample	Range TAN Efficiency	Average TAN Efficiency	s TAN Efficiency	Range NO_2-N Efficiency	Average NO_2-N Efficiency	s NO_2-N Efficiency	Range O_2 Efficiency	Average O_2 Efficiency	s O_2 Efficiency
Sand (2)	17	73.04 to 86.46	80.18	2.89	-78.84 to 53.30	-8.07	40.55	31.36 to 61.96	45.96	9.19
Mn (2)	17	90.11 to 98.33	95.23	1.72	30.16 to 95.28	69.08	21.35	36.40 to 72.82	54.97	11.41
Sand (4)	3	82.79 to 86.36	84.32	1.84	-34.39 to -3.41	-14.72	17.10	57.28 to 59.18	58.08	0.98
Mn (4)	3	96.10 to 96.72	96.43	0.31	66.14 to 74.15	71.48	4.62	64.08 to 68.35	66.3	2.14
Sand (6)	3	89.34 to 90.91	90.13	0.78	5.96 to 23.66	14.11	8.93	55.71 to 60.89	58.93	2.81
Mn (6)	3	96.10 to 96.48	96.71	0.75	78.84 to 83.49	81.33	2.34	62.50 to 68.72	66.58	3.53
Sand (24)	26	75.00 to 95.45	90.61	4.58	-54.70 to 73.43	26.68	35.00	26.88 to 75.65	50.16	15.23
Mn (24)	26	85.71 to 96.97	94.47	2.70	24.04 to 98.06	74.14	21.79	26.88 to 80.50	52.64	16.28

Table 29: Observations from pilot filters carried out 2, 4, 6 and 24 hours after filter backwashing.

4.3.3 Affect of reduced contact time on filter performance

Although nitrification rates depend strongly on substrate concentrations, various authors measured higher ammonia removal rates (g m^3 day^{-1}) at low ammonia concentrations and higher flow rate (shorter retention time), suggesting that retention time was the primary factor affecting removal (Muir, 1982). Liao and Mayo (1974) found a direct relation between ammonia removal and media retention time at various ammonia loading rates. During this stage of the trial, to test the effect of reduced contact time on ammonia and nitrite removal rate, the medium volume in each filter bed was reduced to half with a resulting reduction in the EBCT to one minute.

NH_4^+, NO_2^- and O_2 data were collected over one-months period, measurements were carried out 2, and 24 hours after filter backwashing to compare ammonia and nitrite removal efficiency and removal rate between the sand and MnO_2 pilot filters. During this time the following range of ambient conditions were observed (Table 30).

	Sample No.	Range	Average
Temperature (^{o}C)	12	7.30 - 11.1	8.39
pH	12	6.79 - 6.97	6.88
Alkalinity (mg L^{-1} as $CaCO_3$)	4	26.00 - 50.00	38.00
Dissolved Oxygen (mg L^{-1})	12	9.48 - 13.18	11.57
TSS (mg L^{-1})	4	1.00 - 2.00	1.90
BOD_5 (mg L^{-1}as O_2)	4	10.20 - 14.10	13.70
TAN (mg L^{-1}as Nitrogen)	12	0.46 - 1.00	0.77
Nitrite (mg L^{-1} as Nitrogen)	12	0.67 - 0.178	0.11

Table 30: Range of ambient conditions when the medium volume in each filter bed was reduced to half with a resulting reduction in the EBCT to one minute.

Measurements carried out 24 hours after filter back washing

	TAN in mg L^{-1}			[NO_2-N] in mg L^{-1}			[O_2] in mg L^{-1}				
	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	pH	Temp.(^{o}C)
Sample 1-(Week 20)	0.96	0.27	0.17	0.14	0.18	0.10	9.86	6.90	6.30	6.87	10.50
Sample 2-(Week 20)	1.00	0.33	0.16	0.17	0.20	0.11	10.05	6.78	6.05	6.95	9.90
Sample 3-(Week 21)	1.00	0.38	0.22	0.15	0.19	0.11	11.05	7.95	7.32	6.97	8.00
Sample 4-(Week 23)	0.71	0.22	0.16	0.09	0.14	0.08	11.77	9.10	8.61	6.83	7.60
Sample 5-(Week 23)	0.60	0.08	0.04	0.07	0.10	0.05	11.97	9.73	9.69	6.89	7.90
Sample 6-(Week 24)	0.46	0.17	0.06	0.07	0.11	0.04	12.20	10.20	10.20	6.91	7.40
Sample 7-(Week 24)	0.61	0.15	0.07	0.07	0.12	0.06	13.18	11.02	11.12	6.83	7.30
Average	0.76	0.23	0.13	0.11	0.15	0.08	11.44	8.81	8.47	6.89	8.37
s	0.22	0.11	0.07	0.04	0.04	0.03	1.20	1.65	1.98	0.05	1.29
Removal Efficiency (in %)		70.04	83.52		-38.30	28.59		22.98	25.96		
Removal Rate (g m^{3} day^{-1})		669.02	797.81		-51.52	38.46		3291.43	3718.96		

Table 31: Observations from biologically active pilot filters carried out at one minute EBCT, 24 hours after backwashing.

From the measurements carried out 24 hrs after backwashing, the MnO_2 pilot filter showed a significantly higher ammonia and nitrite removal efficiency, at an average 83.52% for ammonia and 28.59% for nitrite compared to 70.04% for ammonia and – 38.30% (output) for nitrite for the sand filter (Table 31). However, when compared

with observations carried out using twice the media (two minutes contact time), on average a drop in ammonia and nitrite removal efficiency was found for both filters (Table 31). This was probably the result of reducing contact time to one minute. Furthermore, higher ammonia removal rates (g m^3 day^{-1}) at lower average $[NH_4]_{INLET}$ for both filters was found compared with observations carried out using twice the media, suggesting that retention time was the primary factor affecting removal (Table 32).

	Sand	Mn	Sand	Mn
Average $[NH_4]_{INLET}$ (mg L^{-1})	0.9	0.9	0.76	0.76
Average $[NO_2]_{INLET}$ (mg L^{-1})	0.16	0.16	0.11	0.11
EBCT (min.)	2	2	1	1
Average Ammonia Removal Efficiency (%)	91.10	94.76	70.04	83.52
Average Ammonia Removal Rate (g m^3 day^{-1})	510.50	530.97	669.02	797.81
Average Nitrite Removal Efficiency (%)	24.86	76.04	-38.30	28.59
Average Nitrite Removal Rate (g m^3 day^{-1})	24.85	76.02	-51.52	38.46

Table 32: Observations from the pilot filters carried out 24 hours after filter backwashing. Comparison between nitrification performance for MnO_2 and sand pilot filters at different EBCT time.

Figure 33 compares the relationship between $[NH_4]_{INLET}$ and ammonia removal for the two pilot filters. The correlation coefficient between these parameters was good for the sand filter (R^2_{SAND} = 0.8644) and high for MnO_2 filter ($R^2_{MnO_2}$ = 0.9607). Increased removal was found with increased of $[NH_4]_{INLET}$. As this did not exceed 1.00 mg L^{-1} TAN (Figure 33), maximum removal rates were not likely to have been obtained as the graphs indicates it occurs at higher $[NH_4]_{INLET}$. Comparison with observations carried out using twice the media volume (sample 14 – week 11 in Table 24 and

Figure 27), shows that on average the difference in removal of the sand compared to that of the MnO_2 pilot filter increases proportionally more with the increasing $[NH_4]_{INLET}$; the maximum differential observed between the two media was 0.17 mg L^{-1} TAN at $[NH_4]_{INLET}$ of 1.00 mg L^{-1} TAN (sample 2 – week 20 in Table 31 and Figure 33).

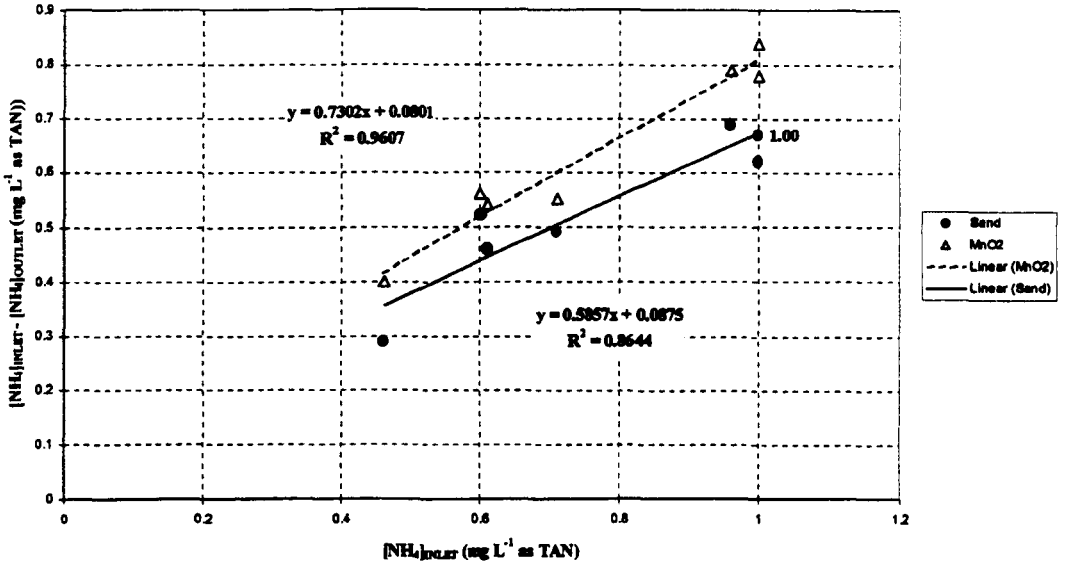


Figure 33: Observations from biologically active pilot filters carried out at one minute EBCT, 24 hours after backwashing. Ammonia removal ($[NH_4]_{INLET} - [NH_4]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

With these smaller volume pilot trials, a considerable drop in nitrite removal efficiency was found on average for both filters. This had the effect of producing a continuous net output of nitrite from the sand filter at all $[NH_4]_{INLET}$ values (Table 29). Figure 34 shows the relationship between $[NH_4]_{INLET}$ and NO_2 removal 24 hours after backwashing. The correlation coefficient between these parameters was very low for the sand filter ($R^2_{SAND} = 0.038$) but a higher correlation was found for the MnO_2 filter

($R^2_{MnO_2}$ = 0.723), where an increased ($[NO_2]_{INLET} - [NO_2]_{OUTLET}$) was found with increasing of $[NH_4]_{INLET}$.

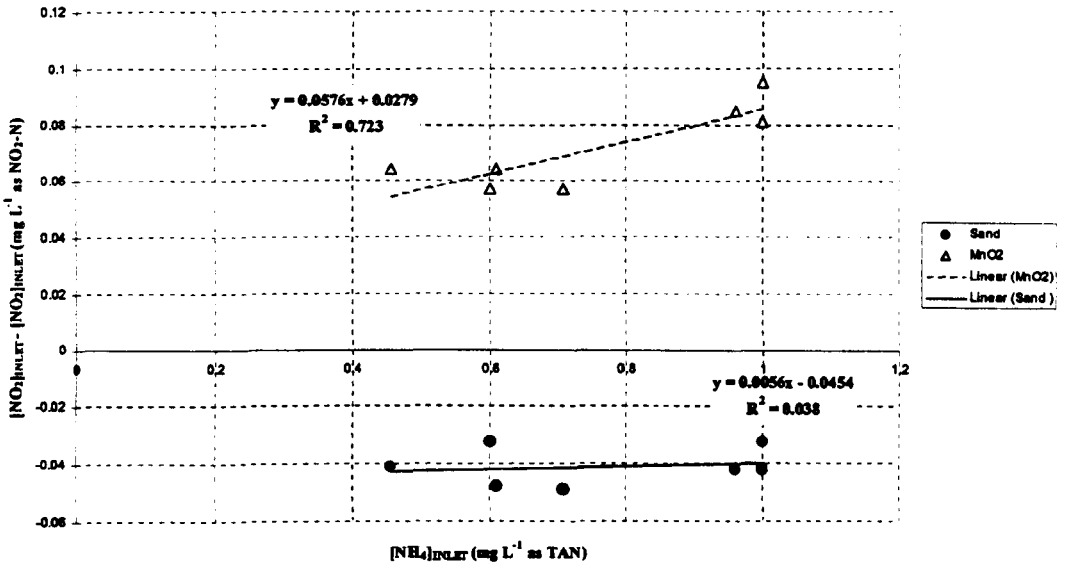


Figure 34: Observations from biologically active pilot filters carried out at one minute EBCT, 24 hours after backwashing. Absolute nitrite removal ($[NO_2]_{INLET} - [NO_2]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

Measurements of oxygen removal for both pilot filters were carried out during the 4-week period. Figure 35 shows the relationship between $[NH_4]_{INLET}$ and O_2 uptake for the two filters. The correlation coefficient between these parameters was high (R^2_{SAND} = 0.9523; $R^2_{MnO_2}$ = 0.9173) for both filters (Figure 35). As expected, increased uptake was found with increased $[NH_4]_{INLET}$. Furthermore, when compared with observations carried out using twice the media volume (sample 14 – week 11 in Table 24 and Figure 29), on average the difference in uptake between the sand and MnO_2 filters increases proportionally more with the increasing $[NH_4]_{INLET}$; the maximum

differential observed between the two media was 0.73 mg L^{-1} O_2 at $[NH_4]_{INLET}$ of 1.00 mg L^{-1} TAN (sample 2 – week 20 in Table 31 and Figure 35).

This seems to confirm that the difference in ammonia removal between the sand and MnO_2 pilot filters increases proportionally more with the increasing $[NH_4]_{INLET}$ (Figure 30) when the contact time is reduced to one minute. Moreover, Figure 33 shows that O_2 uptake regression lines for sand and MnO_2 filters cross, suggesting that below a certain $[NH_4]_{INLET}$ (< 0.52 mg L^{-1} as TAN) the bacterial O_2 usage becomes greater for the sand filter compared to that of the MnO_2 filter.

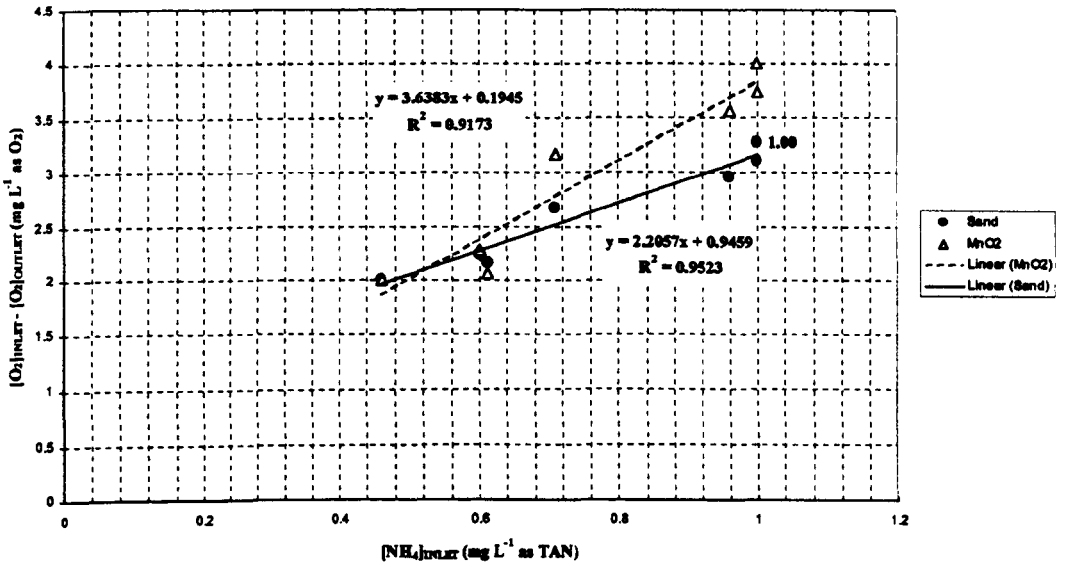


Figure 35: Observations from biologically active pilot filters carried out at one minute EBCT, 24 hours after backwashing. Oxygen removal ($[O_2]_{INLET} - [O_2]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$ for MnO_2 and sand pilot filters.

Measurements carried out 2 hours after back washing

At 2 hrs after filter backwashing, the MnO_2 filter showed a significantly higher ammonia and nitrite removal efficiency; with an average removal efficiency of 74.29% for ammonia and 18.20% for nitrite compared to 59.90% for ammonia and – 24.31% (output) for nitrite of sand filter (Table 33). However, when compared with measurements made 24 hours after backwashing, on average a drop in ammonia removal efficiency was found for both types of filters and in particular, a significant drop in nitrite removal efficiency was found for the MnO_2 filter (Table 31).

In addition, when compared with observations made 2 hours after backwashing but using twice the media (two minutes contact time) (Table 25), on average a drop in ammonia and nitrite removal efficiency was found for both filters, but a higher ammonia removal rate (g m^3 day^{-1}) at lower average $[NH_4]_{INLET}$ for both filters was found, confirming that nitrification rate is affected by retention time (Table 34).

	TAN in mg L^{-1}			[NO_2-N] in mg L^{-1}			[O_2] in mg L^{-1}				
	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	Inlet	Outlet Sand	Outlet MnO_2	pH	Temp.(°C)
Sample 1- (Week 20)	1.00	0.47	0.33	0.18	0.23	0.14	9.48	6.70	5.75	6.87	11.10
Sample 2- (Week 20)	0.83	0.35	0.22	0.13	0.15	0.10	11.00	8.35	7.80	6.95	8.20
Sample 3- (Week 21)	0.80	0.17	0.12	0.08	0.13	0.08	12.00	9.85	9.70	6.79	7.50
Sample 4- (Week 23)	0.65	0.23	0.11	0.08	0.13	0.08	11.70	9.67	9.59	6.88	8.10
Sample 5- (Week 24)	0.61	0.34	0.22	0.08	0.13	0.09	12.10	10.00	9.80	6.82	7.30
Average	0.78	0.31	0.20	0.11	0.14	0.09	11.70	9.47	9.22	6.86	8.44
s	0.16	0.12	0.09	0.04	0.04	0.02	1.08	1.40	1.76	0.06	1.54
Removal Efficiency (in %)		59.90	74.29		-24.31	18.20		19.08	21.18		
Removal Rate (g m^3 day^{-1})		583.51	723.76		-33.12	24.79		2795.48	3102.26		

Table 33: Observations from pilot filters carried out at one minute EBCT, 2 hours after backwashing.

	Sand	Mn	Sand	Mn
Average $[NH_4]_{INLET}$ (mg L^{-1})	1.06	1.06	0.78	0.78
Average $[NO_2]_{INLET}$ (mg L^{-1})	0.19	0.19	0.11	0.11
EBCT (min.)	2	2	1	1
Average Ammonia Removal Efficiency (%)	80.18	95.23	59.90	74.29
Average Ammonia Removal Rate (g m^3 day^{-1})	531.83	631.65	583.51	723.76
Average Nitrite Removal Efficiency (%)	-8.07	69.08	-24.31	18.20
Average Nitrite Removal Rate (g m^3 day^{-1})	-9.57	81.91	-33.12	24.79

Table 34: Observations from biologically active MnO_2 and sand pilot filters carried out 24 hours after backwashing. Comparison between nitrification performance for pilot filters at different EBCT time.

Figure 36 shows the relationship between $[NH_4]_{INLET}$ and ammonia removal for the two pilot filters. The correlation coefficient between these parameters was low for both filters ($R^2_{SAND} = 0.4727$; $R^2_{MnO_2} = 0.6714$).

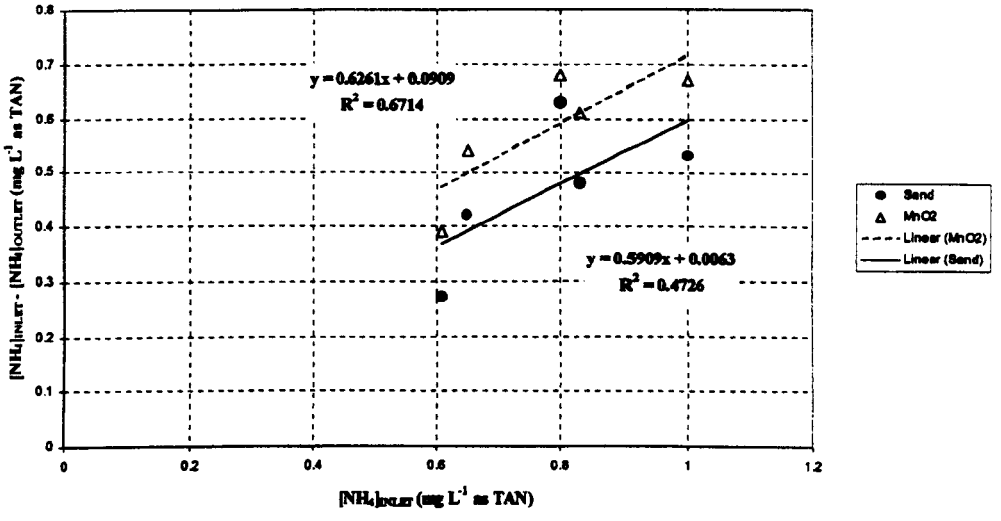


Figure 36: Observations from biologically active MnO_2 and sand pilot filters carried out at one minute EBCT, 2 hours after backwashing. Ammonia removal ($[NH_4]_{INLET} - [NH_4]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$.

Figure 37 shows the relationship between $[NH_4]_{INLET}$ and NO_2 removal for the two filters. The correlation coefficient between these parameters was very low for the sand filter ($R^2_{SAND} = 0.0008$) but increased removal was found with increased $[NH_4]_{INLET}$ for MnO_2 ($R^2_{MnO_2} = 0.7593$). At an average TAN of 0.78 mg L^{-1}, the average NO_2-N output from the sand filter was 0.03 mg L^{-1} compared with a removal of 0.02 mg L^{-1} from the MnO_2 filter (Figure 37).

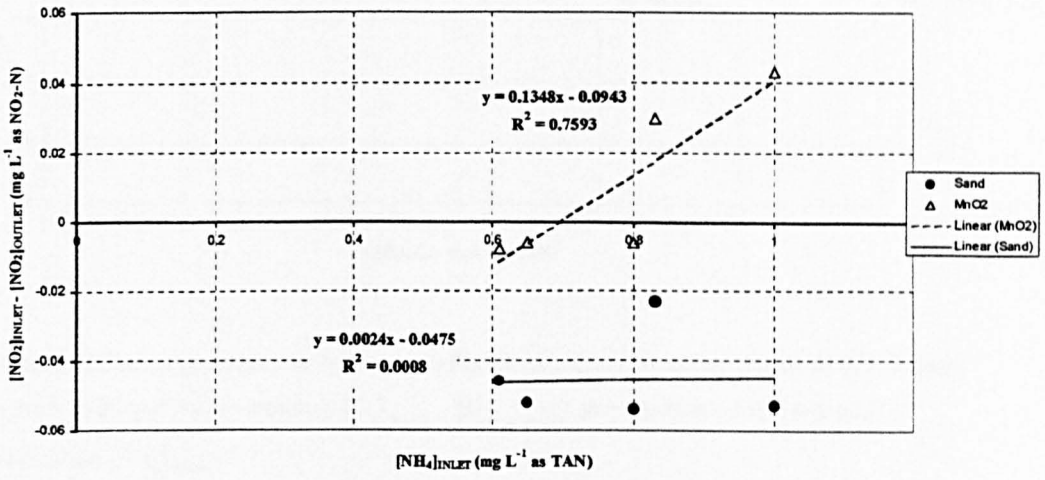


Figure 37: Observations from biologically active MnO_2 and sand pilot filters carried out at one minute EBCT, 2 hours after backwashing. Absolute nitrite removal ($[NO_2]_{INLET} - [NO_2]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$.

Figure 38 shows the relationship between $[NH_4]_{INLET}$ and O_2 uptake showing good correlation for both filters ($R^2_{SAND} = 0.7724$; $R^2_{MnO_2} = 0.7875$). As expected, increased uptake was found with increased $[NH_4]_{INLET}$. The difference in uptake between the sand and MnO_2 filters increases with increasing $[NH_4]_{INLET}$; the maximum differential

observed between the two media was 0.95 mg L^{-1} O_2 at $[NH_4]_{INLET}$ of 1.00 mg L^{-1} TAN (sample 1 – week 20 in Table 33 and Figure 36). Furthermore, the O_2 uptake regression lines for sand and MnO_2 filters suggest that they will cross at low $[NH_4]_{INLET}$ (Figure 38) and follows what already discussed above for Figure 35.

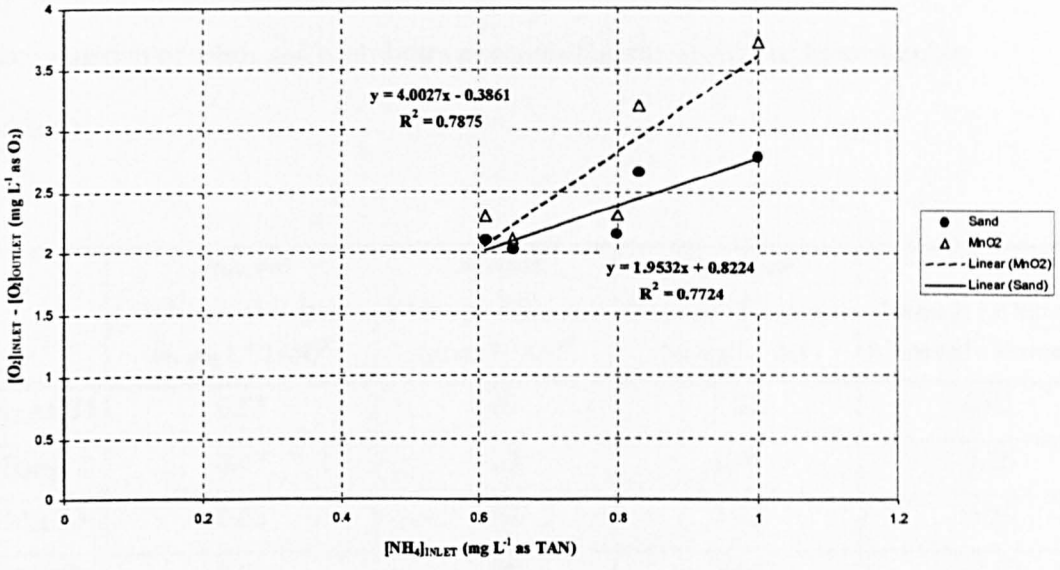


Figure 38: Observations from MnO_2 and sand pilot filters carried out at one minute EBCT, 2 hours after backwashing. Oxygen removal ($[O_2]_{INLET} - [O_2]_{OUTLET}$) as a function of inlet ammonia concentration $[NH_4]_{INLET}$.

From the measurements made 24 and 2 hrs after backwashing the filters over a 4-weeks period, data in Table 35 compares the theoretical and observed O_2 uptake for sand and MnO_2 filters at EBCT of 1 minutes. This was found to exceed theoretical oxygen requirements for ammonia removal for both filters (Table 35) suggesting that a modest degree of heterotrophic activity was present in both filters. However, when compared with observations carried out using twice the media (two minutes contact time) (Table 26), on average a reduction in the difference between theoretical and observed O_2 uptake was measured for both filters. These results suggest that a higher

quantity of oxygen was used from both filters, which did not involve the nitrification process, during the filter trial at two minutes contact time.

Furthermore, the difference between theoretical and observed O_2 uptake decreased as a result of backwashing the filters (Table 35). Backwashing the filter decreases the accumulation of solids and contributes to controlling the growth of heterotrophic bacteria.

	Observed $[NH_4]_{INLET} - [NH_4]_{OUTLET}$ (as mg L^{-1} TAN)[A]	Observed $[O_2]_{INLET} - [O_2]_{OUTLET}$ (as mg L^{-1} O_2)[B]	Theoretical $[O_2]_{INLET} - [O_2]_{OUTLET}$ (as mg L^{-1} O_2)[C]	$[O_2]_{INLET} - [O_2]_{OUTLET}$ (Observed - Theoretical)
Sand 24	0.53	2.63	2.22	0.41
Sand 2	0.47	2.23	1.98	0.25
Mn 24	0.63	2.97	2.72	0.25
Mn 2	0.58	2.48	2.50	-0.02

[A] *Average ($[NH_4]_{INLET} - [NH_4]_{OUTLET}$) over 4 weeks-period for both filters measured 24 and 2 hours after backwashing (Table 24 and 25).*

[B] *Average ($[O_2]_{INLET} - [O_2]_{OUTLET}$) over 4 weeks-period for both filters measured 24 and 2 hours after backwashing (Table 24 and 25).*

[C] *Theoretical ($[O_2]_{INLET} - [O_2]_{OUTLET}$) calculation:*

From the stoichiometry of Eqs (8) and (9) in section 1.3.1 is possible to determine the nitrifying bacterial oxygen requirements: for every gram of NH_4^+ oxidized to NO_3^-, 3.158 g of O_2 are used by Nitrosomonas to convert NH_4^+ to NO_2^- and 1.114 g of O_2 are used by Nitrobacter to convert the produced NO_2^- to NO_3^-. Total O_2 consumption for 1 g of NH_4^+ oxidized to NO_3^- is equal to 4.273 g (3.158 g + 1.114 g). Moreover, for every gram of $[NO_2]_{INLET}$ oxidized to NO_3^-, 0.350 g of O_2 is used by Nitrobacter to convert NO_2^- to NO_3^-.

The following method was used to calculate the theoretical ($[O_2]_{INLET} - [O_2]_{OUTLET}$):

$(([NH_4]_{INLET} - [NH_4]_{OUTLET}) \times 4.273) + (([NO_2]_{INLET} - [NO_2]_{OUTLET}) \times 1.114)$

Table 35: Comparison between theoretical and observed ($[O_2]_{INLET} - [O_2]_{OUTLET}$) for MnO_2 and sand pilot filters.

4.3.4 Manganese solubility

Manganese solubility was measured by monitoring the change in total manganese concentration $[Mn]_{TOTAL}$ as recycled water passed through the MnO_2 filter. Measurements were carried out over a 24-week-period. During this time the following range of ambient conditions was observed (Table 36).

	Sample No.	Range	Average
Temperature (°C)	69	7.30 - 17.00	12.51
pH	69	6.79 - 7.40	7.04
Alkalinity (mg L^{-1} as $CaCO_3$)	26	26.00 - 50.00	38.00
Dissolved Oxygen (mg L^{-1})	69	8.40 - 13.18	10.37
TSS (mg L^{-1})	26	1.00 - 2.00	1.53
BOD_5 (mg L^{-1}as O_2)	26	8.70 - 15.20	12.47
TAN (mg L^{-1}as Nitrogen)	69	0.08 - 1.66	0.65
Nitrite (mg L^{-1} as Nitrogen)	69	0.02 - 0.26	0.11

Table 36: Range of ambient conditions during the 24 week period of the filters trial.

Data in Table 37 shows that on average, in the range of ambient conditions tested (Table 36), an output of $[Mn]_{TOTAL}$ was not detected from samples collected 24 and 2 hours after backwashing. On a few occasions a small increase in $[Mn]_{TOTAL}$ at the outlet of the filter was measured (Week 2, 9, 12, 14, 15, 21, 23); the $[Mn]_{TOTAL}$ increase was found both 24 and 2 hrs after backwashing and did not exceed 2 $\mu g\ L^{-1}$. However, since these values are close to the lowest limit of the instrument sensitivity, they should be viewed with caution (Struthers, personal communication).

	24 hours after backwashing		2 hours after backwashing	
	Total Mn Inlet (ug L^{-1})	Total Mn Outlet (ug L^{-1})	Total Mn Inlet (ug L^{-1})	Total Mn Outlet (ug L^{-1})
Week 1 in Table 19	4	3	n/a	n/a
Week 2 in Table 19	3	5	n/a	n/a
Week 3 in Table 19	4	4	n/a	n/a
Week 4 in Table 19	4	4	n/a	n/a
Week 5 in Table 21	4	4	4	4
Week 6 in Table 21	3	3	5	3
Week 7 in Table 21	4	4	3	3
Week 8 in Table 21	4	3	5	3
Week 9 in Table 21	4	3	3	4
Week 10 in Table 21	4	4	5	4
Week 11 in Table 21	5	5	4	2
Week 12 in Table 21	4	4	3	4
Week 13 in Table 21	4	4	5	3
Week 14 in Table 21	3	4	3	3
Week 15 in Table 21	4	4	3	5
Week 16 in Table 21	3	3	3	3
Week 17 in Table 21	4	4	4	3
Week 18 in Table 21	4	4	2	2
Week 19 in Table 21	3	3	2	2
Week 20 in Table 28	4	3	2	2
Week 21 in Table 28	3	2	2	3
Week 22 in Table 28	3	3	3	3
Week 23 in Table 28	2	3	1	2
Week 24 in Table 28	3	3	3	3
Average	**3.63**	**3.58**	**3.25**	**3.05**

Table 37: Manganese solubility.

Chapter 5
Technical and financial analysis

5.1 Introduction

The commercial scale field trials point out the potential of manganese dioxide as a biological media to remove ammonia and nitrite in RAS. The results, though obtained from a limited number of replicates, suggest that manganese dioxide could have some operating advantages when compared with silicate sand commonly used in biological filters. The following observations were apparent, where the MnO_2 medium:

- is more efficient at removing ammonia as the $[NH_4]_{INLET}$ increases and the retention time decreases.
- is more efficient at removing nitrite, showing a more reliable capacity to complete the ammonia oxidation to nitrate. The silicate sand media accumulated nitrite as $[NH_4]_{INLET}$ increases and the retention time decreases.
- did not appear to be soluble in the ambient conditions encountered.

Based on improving technical performance of the commercial scale hatchery, as highlighted during the fieldwork, a technical and financial analysis was developed to investigate further the usage of manganese dioxide as a biological media to remove ammonia and nitrite in RAS.

5.1.1 Commercial scale RAS technical background

As described in chapter 4, the RAS layout includes a main and a side stream water treatments with a central sump (Figure 24). The 'main stream' incorporates 8 biological trickling filter units, which represent the main biological process in the recirculation system. Their technical characteristics are presented in Table 38.

Horizontal filter surface (m^2)	4.50
Unit volume (m^3)	9
Number of units	8
Total horizontal surface (m^2)	36
Total volume (m^3)	72
Filter media	3.5 in. koch rings
Specific surface area (m^2/m^3)	220
Bio-filter surface (m^2)	1980
Total surface (m^2)	15840
Void fraction filter media (1)	0.92
Flow rate (m^3/h)	432
Media retention time (hr) (2)	0.15
Hydraulic loading rate (m^3/m^2d)	0.65
Hydraulic loading rate (m^3/m^3d)	144

(1) Ratio between empty volume and total volume

(2) Media retention time = (Total media volume x Void fraction)/ Flow rate

Table 38: Trickling filter technical characteristics.

During the 24 weeks of the pilot filter trials, measurements were carried out from four different trickling filter units operating in the recirculation system (units 3, 4, 5 and 6). The aim was to evaluate the performance with respect to ammonia and nitrite conversion to nitrates. Samples were collected once a week from the inlet and outlet of each trickling filter unit. Temperature, pH, ammonia and nitrite were tested from the water samples. Temperature and pH were measured with WTW pH 320

instrument. Samples of ammonia, nitrite were measured with a spectrophotometer water analysis kit (Palintest 5000).

The trickling filter measurements were carried out during a phase of the production cycle when the biomass into the system fluctuated from 1000 kg to a maximum of 15,000 kg with a feed input ranged from 50 kg day^{-1} to a maximum 250 kg day^{-1}. As a result the system ammonia and nitrite concentration fluctuated during this period (Figure 39). In particular, Figure 39 shows that between week 9 and week 12 there was a considerable drop in metabolite concentration, which was mainly caused by the first and most extended fish grading of the production cycle where fish are not fed. From week 16 onwards, the decline in metabolite concentration was simply caused by ambient temperature drop, which reduced the feed input in the system (Table 39).

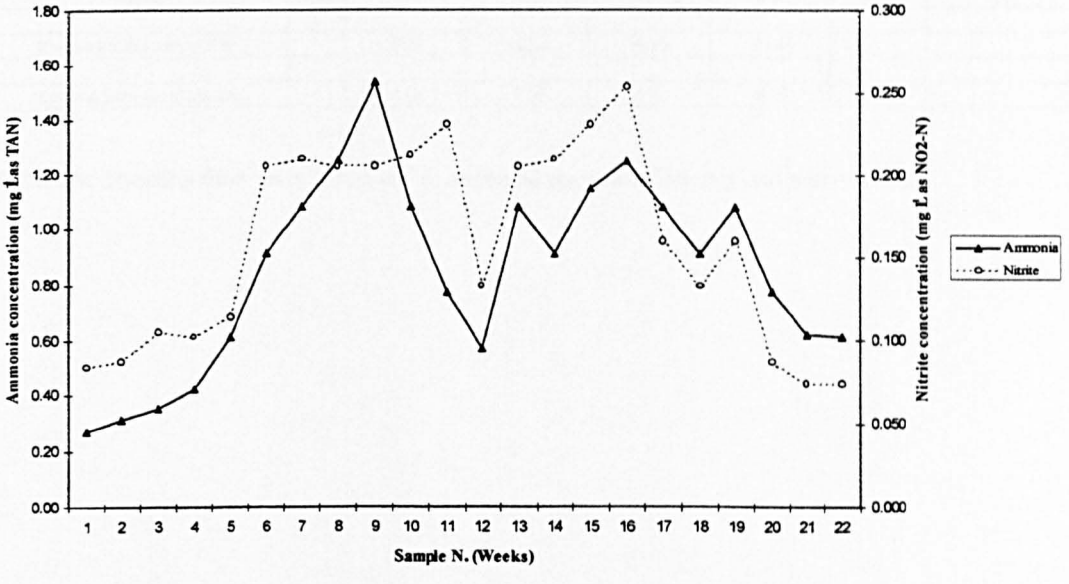


Figure 39: The fluctuations of ammonia and nitrite concentration at Quoys RASs.

Typical performances with respect to ammonia and nitrite removal efficiencies and removal rates of the trickling filter units are presented in Table 39 and 40.

Nitrification rates of Quoys hatchery trickling filters (Table 39 and Figure 40) were in general agreement with observed ammonium removal rate found in literature (van Rijn and Rivera, 1990; Nijhof, 1995; Kamstra *et al.*, 1998).

	[NH4-N] in mg/L						
	Inlet	Outlet Filter 3	Outlet Filter 4	Outlet Filter 5	Outlet Filter 6	pH	Temperature (°C)
Sample 1-(Week 3)	0.27	0.16	0.21	0.20	0.21	7.27	14.20
Sample 2-(Week 4)	0.31	0.14	0.24	0.23	0.23	7.22	15.30
Sample 3-(Week 5)	0.35	0.20	0.22	0.20	0.24	7.19	16.50
Sample 4-(Week 6)	0.42	0.27	0.34	0.30	0.25	7.40	16.50
Sample 5-(Week 7)	0.61	0.45	0.43	0.42	0.50	7.00	16.10
Sample 6-(Week 8)	0.91	0.64	0.71	0.68	0.70	7.00	15.90
Sample 7-(Week 9)	1.08	0.68	0.77	0.71	0.70	7.07	16.10
Sample 8-(Week 10)	1.25	0.91	0.91	0.96	0.96	6.96	16.00
Sample 9-(Week 11)	1.54	1.15	1.08	1.15	1.00	6.81	14.90
Sample 10-(Week 12)	1.08	0.64	0.70	0.64	0.71	6.90	14.30
Sample 11-(Week 13)	0.77	0.51	0.52	0.51	0.47	6.92	13.90
Sample 12-(Week 14)	0.57	0.42	0.45	0.47	0.48	7.18	13.30
Sample 13-(Week 15)	1.08	0.71	0.61	0.61	0.70	6.86	13.10
Sample 14-(Week 16)	0.91	0.68	0.64	0.70	0.68	6.88	13.00
Sample 15-(Week 17)	1.15	0.71	0.64	0.70	0.71	6.81	12.80
Sample 16-(Week 18)	1.25	0.96	1.00	0.96	0.91	6.77	12.30
Sample 17-(Week 19)	1.08	0.70	0.71	0.64	0.70	6.80	9.40
Sample 18-(Week 20)	0.91	0.64	0.71	0.68	0.70	6.90	10.20
Sample 19-(Week 21)	1.08	0.61	0.77	0.71	0.70	7.00	8.10
Sample 20-(Week 22)	0.77	0.47	0.52	0.51	0.51	6.98	8.00
Sample 21-(Week 23)	0.62	0.48	0.54	0.57	0.58	6.89	7.90
Sample 22-(Week 24)	0.61	0.45	0.43	0.42	0.50	6.84	7.30
Average	0.85	0.57	0.60	0.59	0.60	6.90	8.48
Removal Efficiency (in %)		32.48	29.39	30.40	29.46		
Removal Rate (in g/m2/day)		0.18	0.16	0.17	0.16		

Table 39: Trickling filter units 3, 4, 5 and 6; ammonia removal efficiency and removal rate.

	[NO2-N] in mg/l						
	Inlet	Outlet Filter 3	Outlet Filter 4	Outlet Filter 5	Outlet Filter 6	pH	Temperature (°C)
Sample 1-(Week 3)	0.084	0.096	0.096	0.093	0.099	7.27	14.20
Sample 2-(Week 4)	0.087	0.093	0.084	0.105	0.105	7.22	15.30
Sample 3-(Week 5)	0.105	0.124	0.121	0.111	0.111	7.19	16.50
Sample 4-(Week 6)	0.102	0.118	0.118	0.121	0.121	7.40	16.50
Sample 5-(Week 7)	0.114	0.121	0.128	0.115	0.132	7.00	16.10
Sample 6-(Week 8)	0.205	0.231	0.231	0.254	0.238	7.00	15.90
Sample 7-(Week 9)	0.209	0.238	0.231	0.238	0.231	7.07	16.10
Sample 8-(Week 10)	0.205	0.231	0.238	0.231	0.231	6.96	16.00
Sample 9-(Week 11)	0.205	0.231	0.254	0.242	0.238	6.81	14.90
Sample 10-(Week 12)	0.212	0.231	0.231	0.231	0.238	6.90	14.30
Sample 11-(Week 13)	0.231	0.254	0.238	0.254	0.231	6.92	13.90
Sample 12-(Week 14)	0.132	0.173	0.173	0.173	0.171	7.18	13.30
Sample 13-(Week 15)	0.205	0.231	0.231	0.238	0.231	6.86	13.10
Sample 14-(Week 16)	0.209	0.254	0.231	0.254	0.238	6.88	13.00
Sample 15-(Week 17)	0.231	0.254	0.254	0.238	0.280	6.81	12.80
Sample 16-(Week 18)	0.254	0.280	0.280	0.254	0.254	6.77	12.30
Sample 17-(Week 19)	0.160	0.212	0.205	0.205	0.212	6.80	9.40
Sample 18-(Week 20)	0.132	0.189	0.173	0.173	0.171	6.90	10.20
Sample 19-(Week 21)	0.160	0.209	0.205	0.205	0.212	7.00	8.10
Sample 20-(Week 22)	0.087	0.093	0.084	0.105	0.093	6.98	8.00
Sample 21-(Week 23)	0.074	0.069	0.069	0.074	0.072	6.89	7.90
Sample 22-(Week 24)	0.074	0.069	0.069	0.074	0.072	6.84	7.30
Average	0.16	0.18	0.18	0.18	0.18	6.90	8.48
Removal Efficiency (in %)		-15.07	-13.43	-14.70	-14.50		
Removal Rate (in g/m2/day)		-0.02	-0.01	-0.02	-0.01		

Table 40: Trickling filter units 3, 4, 5 and 6; nitrite removal efficiency and removal rate.

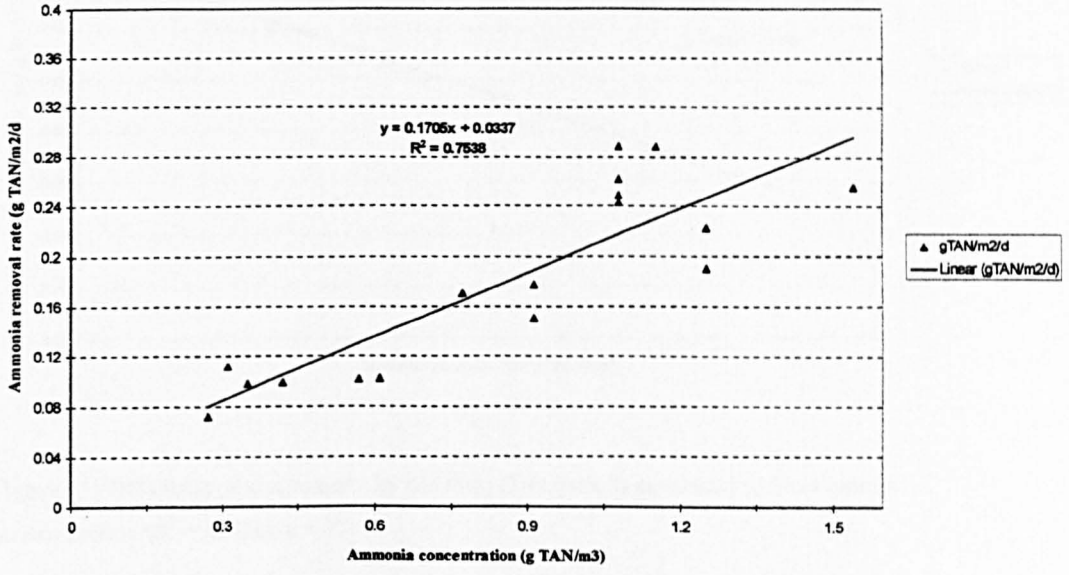


Figure 40: Ammonia removal rates by the trickling filter (unit 3) at various ambient ammonia concentrations ($R^2 = 0.7538$, n = 22).

Increased removal of ammonia by the trickling filters was found with increasing ambient concentrations of ammonia. At an average $[NH_4]_{INLET}$ = 0.85 mg L^{-1} the total ammonia removal/day was based on the following calculation (see Table 39 and Figure 40):

Removal/day = Average Removal rate (g/TAN/m^2) x Total surface (m^2) =

= 0.17 g/TAN/m^2 x 15840 m^2 = 2693 g of TAN/day

However, as reported by various authors (Poxton *et al.*, 1981; Bovendeur *et al.*, 1987; van Rijn and Rivera, 1990; Nijhof and Klapwijk, 1995; Kamstra *et al.*, 1998), a net production of nitrite was detected (Table 40 and Figure 41).

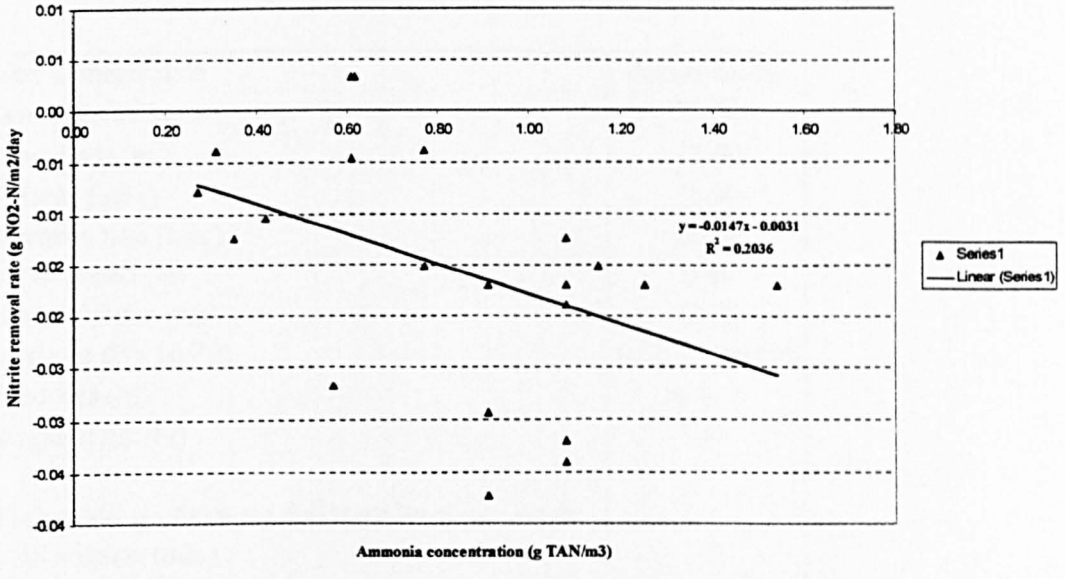


Figure 41: Net nitrite production rate by trickling filter (unit 3) at various ambient ammonia concentrations (R^2 = 0.2036, n = 22).

As a result of the partial oxidation of ammonia to nitrate, increased net nitrite production rate was found with increasing ambient concentrations of ammonia. On

average $[NH_4]_{INLET}$ = 0.85 mg L^{-1} the net nitrite production/day was based on the following calculation (see Table 40 and Figure 41):

Production/day = Average production rate (g/NO_2-N/m^2) x Total surface (m^2) =

= 0.02 g/TAN/m^2 x 15840 m^2 = 317 g of NO_2-N/day

Furthermore, the hatchery RAS included two submerged rapid-sand filters positioned 'side stream' (Figure 22). They were primarily designed to remove the finer suspended solids after mechanical filtration. However, the results obtained from the pilot filters suggest that sand filtration could have also been an efficient treatment for the removal of ammonia and nitrite. The technical characteristics of the submerged sand filters are presented in Table 41.

Filter Configuration	Mono-media
Flow rate (m^3/hr)	39.60
Size of bed (m^2)	15.00
Velocity (m/hr)	2.64
Retention time (min.)	20.45
Depth of bed (m)	0.90
Media Volume (m3)	13.50
Sand size (BS 16/30)	0.55 - 1.19 mm
Head loss (m)	up to 1
Length of run (hr)	24
Backwashing: Aeration followed by water wash	
Initial aeration (min.)	15
Cleaning water consumption*	5-6% of output
Backwash flow rate (m^3/hr)	100.80
Backwash water velocity (m/hr)	6.72

**Assuming that the backwashing was carried out daily for 30 minutes, the cleaning water consumption was calculated as follows:*
Cleaning water consumption (%) =((100.80 m3/hr x 0.5) / (39.60 m3/hr x 24 hrs)) x 100

Table 41: Submerged gravity sand filter technical characteristics.

Starting from the degree of recycling, feed input per day, process flow characteristics and removal efficiency of the biological treatments, the metabolite mass balance of the RASs was calculated (Table 42) (Lawson, 1995). The removal efficiencies of the trickling filters was obtained from Tables 39 and 40 whereas that for submerged sand filters comes from the results obtained during the field trial (see Table 24). The degree of recycling was high and a minimum of about 2% of the flow rate was exchanged during each turnover (around 180 L min^{-1}) to maintain a steady system volume, which at full capacity reached about 1,000 m^3 (see Table 17). The maximum system feed input was around 250 Kg day^{-1} and in order to minimize fluctuations of the water chemistry environment, the daily amount of feed fed to the fish was given uniformly throughout the 24 hrs cycle.

Feed/Day	250										Kg
V = system volume	1000000										L
Q = flow rate through the system	180										L/min
	8 x Trickling filter	2 x Sand filter									
Qf = flow rate	7200	1200									L/min
Efficiency (NH4-N) to (NO2-N)	0.31	0.91									
Efficiency (NO2-N) to (NO3-N)	0.86	1.25									
Ammonia Mass Balance	Day 1						Day 2				
t = time	**10:00 AM**		**11:00 AM**		**10:00 PM**		**9:00 AM**		**10:00 AM**		min
Ci TAN system	**1.48**		**1.48**		**1.49**		**1.49**		**1.49**		mg/L
PR TAN = production rate (1)	5208										mg/min
CR TAN = consuption rate	3303	1616	3306	1618	3317	1623	3318	1623	3318	1623	mg/min
(dCTAN/dt)*V =	22		18		1		0		0		mg/min
CTAN increase	0.00		0.00		0.00		0.00		0.00		mg/L
Cf TAN system	**1.48**		**1.48**		**1.49**		**1.49**		**1.49**		mg/L
Nitrite Nitrogen Mass Balance											
Ci NO2-N	**0.200**		**0.201**		**0.215**		**0.228**		**0.229**		mg/L
PR NO2-N	3303	1616	3306	1618	3317	1623	3318	1623	3318	1623	mg/min
CR NO2-N	2841	2020	2843	2022	2852	2028	2853	2029	2853	2029	mg/min
(dCNO2/dt)*V	22		22		20		18		17		mg/min
CNO2 system increase	0.001		0.001		0.001		0.001		0.001		mg/L
Cf NO2 system	**0.201**		**0.203**		**0.217**		**0.229**		**0.230**		mg/L

(1) Assuming 0.03 Kg Ammonia / Kg feed.

Table 42: Quoys RASs mass balance predicting the trend of ambient ammonia and nitrite concentration during the 24 hr production cycle.

Table 42 shows that, a theoretical steady ammonia concentration (1.49 mg L^{-1}) was obtained from the mass balance calculation at the maximum system feed input of 250 Kg day^{-1}. This was in agreement with hatchery measurements where at the peak of the feed input TAN reached value of 1.54 mg L^{-1}. Nevertheless, although the ammonia concentration in the system was controlled, it was difficult to predict the levels of nitrite. Table 42 shows that the ambient nitrite concentration increases steadily during the 24 hr production cycle as a result of a higher production rate by the trickling filters, compared with the nitrite consumption rate carried out by the sand filters. Consequently, given that the sand filters were at their maximum flow rate capacity, during the production cycle the only method to control the accumulation of the nitrite in the system was to decrease the degree of recycle (*i.e.* flush through more fresh water) or to reduce the feed input.

In view of the above system setting, the pilot trial results suggest that by simply replacing a proportion of the sand media with manganese dioxide it could be possible to increase the nitrite removal rate from the two submerged sand filters. A standard catalytic filters consist of 80% by volume standard filter sand BS 16/30 grades, and 20% MnO_2 BS 18/44 grades, where two different grades are used in order to create a homogeneous combination of media with different density (UMS Ltd., Personal communication). Assuming that the replacement of 20% of sand BS 16/30 with MnO_2 would increase the nitrite removal efficiency but would not affect the filter flow rate, the following result can be predicted (Table 43).

Feed/Day	250										Kg
V = system volume	1000000										L
Q = flow rate through the system	180										L/min
	Trickling filter	2 x Sand filters									
Qf = flow rate	7200	1200									L/min
Efficiency (NH4-N) to (NO2-N)	0.31	0.95									
Efficiency (NO2-N) to (NO3-N)	0.86	1.76									
Ammonia Mass Balance	Day 1						Day 2				
t = time	10:00 AM		11:00 AM		10:00 PM		9:00 AM		10:00 AM		min
Ci TAN system	1.47		1.47		1.47		1.47		1.47		mg/L
PR TAN = production rate	5208										mg/min
CR TAN = consuption rate	3281	1676	3279	1675	3273	1672	3273	1672	3273	1672	mg/min
(dCTAN/dt)*V =	-13		-10		-1		0		0		mg/min
CTAN increase	0.00		0.00		0.00		0.00		0.00		mg/L
Cf TAN system	1.47		1.47		1.47		1.47		1.47		mg/L
Nitrite Nitrogen Mass Balance											
Ci NO2-N	0.200		0.149		-0.376		-0.842		-0.882		mg/L
PR NO2-N	3281	1676	3279	1675	3273	1672	3273	1672	3273	1672	mg/min
CR NO2-N	2822	2949	2820	2948	2815	2942	2815	2942	2815	2942	mg/min
(dCNO2/dt)*V	-850		-841		-745		-661		-654		mg/min
CNO2 system increase	-0.051		-0.050		-0.045		-0.040		-0.039		mg/L
Cf NO2 system	0.149		0.099		-0.421		-0.882		-0.921		mg/L

Table 43: Quoys RASs mass balance predicting the trend of ambient ammonia and nitrite concentration during the 24 hr production cycle. 20% of the BS 16/30 sand media (0.595 – 1.19 mm) was replaced with BS 18/44 manganese dioxides (0.355 – 1 mm).

Table 43 shows that, the ambient nitrite concentration decreases steadily during the 24 hr production cycle as a result of a higher consumption rate by submerged catalytic filters, compared with the nitrite production rate carried out by the trickling filters. However, to investigate a more general application of manganese dioxide technology in intensive aquaculture system, the theoretical replacement was considered of the complete trickling biofilters with a selected filter configuration to apply manganese dioxide technology. Thus a practical comparison between the use of conventional biological treatments and manganese dioxide technology could be made.

5.2 Technical analysis

The pilot filter results suggest that retention time was a primary factor affecting metabolite removal, where the MnO_2 medium was more efficient then silicate sand at oxidizing ammonia and nitrite as the retention time decreased. Consequently, filter

configurations with low retention time appear to be the most effective for the application of manganese dioxide technology, particularly when systems with low nutrient levels are considered. Moreover, at the present time fluidised-sand beds are the most commonly used biological treatment in intensive aquaculture conditions because they appear to be the most cost-effective (Timmons and Summerfelt, 1998; Summerfelt and Wade, 1998; Courtland, 2000). For these reasons the fluidised bed configuration, with relatively high water velocity and short retention time, was selected for the comparative assessment.

The investigation aimed to assess the manganese dioxide technology through the replacement of the existing biological trickling filters, mainly responsible for nitrite production in the system, with fluidised beds using silicate sand and MnO_2 medias. The aim was to design two biological filters having the same ammonia removal capacity as the existing trickling filters, *i.e.* able to remove 2693 g of TAN/day, but also able to complete the ammonia oxidation to nitrate in order to control ambient nitrite concentration.

The starting point was to highlight the nitrification rates and efficiencies for the two medias. Based on the results of the pilot trials, Table 44 summarized the two different residence time scenarios considered for the design study. Although scenario 1 presented lower nitrification rates compared with scenario 2, the oxidation of ammonia to nitrate was completed together with a net consumption of nitrite (nitrite removal efficiency > 100%). In contrast, in scenario 2 the sand media presented a net nitrite production (nitrite removal efficiency = 62%), which was even greater then that

observed for the trickling filters. Therefore scenario 1 was used for the sand media and scenario 2 was used for the MnO_2 media.

	Scenario 1	Scenario 2
Empty Bed Contact Time (EBCT in min.)	**2.3**	**1.2**
Sand TAN removal efficiency[1] (%)	91	70
Sand TAN removal rate[2] ($g/m^3/day$)	482	745
Sand nitrite removal efficiency (%)	125	62
Sand nitrite removal rate ($g/m^3/day$)	25	-52
Mn TAN removal efficiency (%)	95	83
Mn TAN removal rate ($g/m^3/day$)	507	889
Mn nitrite removal efficiency (%)	176	129
Mn nitrite removal rate ($g/m^3/day$)	76	39

(1) The efficiencies for sand and manganese dioxide medias were obtained from the pilot trials study.

(2) The removal rates were calculated by using the average inlet TAN concentration of 0.85 mg L^{-1} found for the trickling filter and by considering that the removal efficiency for ammonia and nitrite remained constant in the range of ammonia inlet measured.

Table 44: Nitrification rates and efficiencies used to design the biofilters.

From Table 44, the amount of media required to remove 2693 g TAN/day can be worked out for the two different fluidised beds:

Sand filter:

(2693 g TAN/day) / (482 g TAN removed/m^3 media/day) = 5.6 m^3 of sand

Manganese dioxide filter:

(2693 g TAN/day) / (889 g TAN removed/m^3 media/day) = 3.0 m^3 of manganese dioxide

Subsequently, the water velocity required to expand the sand bed was determined (Timmons and Summerfelt, 1998). The authors recommend 50% expansion to assure

constant aerobic condition inside the filter column. Although the water velocity indicated by these researchers was referring to a sand media with grade size 18/30, while the media used for the pilot trials was 16/30, the same water velocity was applied to design the fluidised columns. The water velocity required to expand the MnO_2 bed by 50% was found by extrapolating this value based on the relative densities of manganese dioxide and sand. By knowing the water velocity required for sand grade 18/30 and the density of sand and MnO_2 grade 16/30, the following calculation was carried out:

MnO_2 water velocity* = (Sand water velocity x Mn density)/ Sand density =

= (101 m/hr x 1.956 g/ml)/ 1.695 g/ml = 117 m/hr

The next stage had been to design the fluidised-bed columns where the filter cross section was governed by water velocity and empty bed contact time (EBCT), while the depth of the bed was governed by the required media volume and filter cross section. Because water velocity, EBCT and media volume were fixed, a unique design option was available per each type of medias (Table 45

Scenario	Flow rate (m3/hr)	Water velocity (m/hr)	Cross section (1) (m2)	Depth of the Bed (m)	Expanded Bed (m)	Column height (m)	Media Volume m3	Retention time (min.)
Sand 1	146	**101**	1.5	3.9	5.9	6.9	**5.6**	**2.30**
Mn 2	157	**117**	1.3	2.2	3.3	4.3	**3**	**1.15**

(1) Condition of even flow characteristics was certainly achieved inside the fluidised column.

Table 45: Results from the two scenarios used to design the fluidised beds at 50% bed expansion.

* *The sand and MnO2 had the same grade size (BS 16/30) and it was assumed that particle shape, effective size, uniformity coefficient and mean size (Timmons and Losordo, 1998) were equal for the two media.*

Table 45 shows that, due to the higher ammonia and nitrite removal rates, a lower volume of media and a shorter retention time were required for the manganese dioxide system. These also contributed to reduce the depth of the bed and consequently the total pumping head requirements. Nevertheless, MnO_2 is also heavier requiring a higher water velocity and flow rate comparing with sand media. As a general guideline, the total pressure requirement (in m) is equal to three times the sand depth plus the height that the water must be lifted from the sump to the top of the biofilters (Timmons and Summerfelt, 1998). The total pressure required to expand the MnO_2 media was found by extrapolating this value based on the relative densities of manganese dioxide and sand. The total pumping head can then be worked out for the two different fluidised beds:

Sand filter:

Total pumping head = (media depth x 3) + Column height + sump depth = (3.9 x 3) + 6.9 + 1 = 11.7 + 7.9 = 18.8 m

Manganese filter:

Total pumping head = (media depth x 3.5) + Column height + sump depth = (2.2 x 3.5) + 4.3 + 1 = 7.7 + 5.3 = 13 m

Considering that total pumping head requirements generally range from 5 to 11 m (Timmons and Summerfelt, 1998), a compromise had to be found to reduce the column height and total pumping head, particularly in the case of the sand column. As a result, the filter bed expansions were reduced to 20% (lower water velocity) and the filter design was accordingly modified (Table 46).

Scenario	Flow rate (m3/hr)	Water velocity (1) (m/hr)	Cross section (2) (m2)	Depth of the Bed (m)	Expanded Bed (m)	Column height (m)	Media Volume m3	Retention time (min.)
Sand 1	146	**50**	2.9	1.9	2.3	3.3	**5.6**	**2.30**
Mn 2	157	**58**	2.7	1.1	1.3	2.3	**3.0**	**1.15**

(1) The value for the sand media was found in literature (Timmons and Summerfelt, 1998), while the one for the manganese dioxide was extrapolated from it.

(2) Assuming that condition of even flow characteristics was still maintained inside the column

Table 46: Results from the two scenarios used to design the fluidised beds at 20% bed expansion.

The reduced total pumping head can be worked out for the two fluidised beds:

Sand filter:

Total pumping head = (media depth x 3) + Column height + sump depth = (1.9 x 3) + 3.3 + 1 = 5.7 + 4.3 = 10 m

Manganese filter:

Total pumping head = (media depth x 3.5) + Column height + sump depth = (1.1 x 3.5) + 2.3 + 1 = 3.9 + 3.3 = 7.2 m

To summarise, Table 47 compares the most important technical characteristics selected for the fluidised columns, using MnO_2 and sand, and the trickling biofilters used in the commercial hatchery.

Type of biofilter technology	Fluidised bed	Fluidized bed	Trickling filter
Type of media [1]	Mn (16/30)	Sand (16/30)	3.5 in. kock rings
Volume of media (static) (m^3)	3.1	5.6	72
Cross section (m^2)	2.8	2.9	36
Height of the biofilter (m)	2.3	3.3	3
Volume of the biofilter (m^3)	7.1	10.9	108
Water velocity (m/h) [2]	58	50	12
Contact time with static media (min.)	1.2	2.3	9
TAN removal rate (g/m^3/day) [3]	889	482	37.4
Nitrite removal rate (g/m^3/day) [4]	1147	603	36.5
Flow rate (m^3/hr)	162	146	432
Total pumping head (m) [5]	7.2	10	5
Power required (Kw) [6]	3.5	4.5	12

(1) A (16/30) grain size corresponds to the 0.595 – 1.19 mm size, which was used for the pilot trials.

(2) For the fluidised beds was considered water velocity for a 20% media expansion. For the trickling filter the value was worked out as follows: Flow rate / cross section = 432 m^3/h / 36 m^2 = 12 m/h.

(3) (4) Removal rates for the fluidised beds was obtained from the pilot trials results (Table 40), while trickling filter removal rates were obtained from Quoys hatchery measurements (Table 36 and 37).

(5) The total pumping head for the trickling filters was obtained by considering the height required to lift the water from the sump to the top of the biofilter.

(6) *These values were obtained from a commercial catalogue (Flight pumps 2003) by considering pumps series n. N3127.*

Table 47: Comparison between the technical characteristics of fluidised-manganese dioxide bed, fluidised-sand bed and trickling filters.

As can be observed on Table 47, the trickling filters had the lowest nitrification rates. Consequently, the volume of media required to remove 2693 g TAN/day was much greater then for the fluidised beds, and the trickling biofilter also required a much greater flow rate. Overall, the fluidised beds were estimated to have a power

requirement of about 30% of that for the trickling biofilter. Comparable flow rate were used for the fluidised beds, but as a result of the lower volume of media required for the manganese dioxide, the estimated power consumption was around 20% higher for the fluidised-sand filter.

Table 47 shows that a net production of nitrite was carried out from the trickling biofilter, thereby limiting the commercial system. In contrast, nitrite could be successfully controlled using the fluidised beds. In this case ammonia oxidation to nitrate was completed together also with a net consumption of nitrite. However, the MnO_2 media would appear to be more reliable in oxidising nitrite to nitrate compared with sand as result of the following evidence:

1. Based on the first set of pilot trials - 24 hours after filter backwashing (EBCT = 2.3 min.), on a few occasions sand filter showed a net production of nitrite (Weeks 8, 9 and 11). By applying the average ammonia and nitrite removal efficiency found during these occasions to the commercial system mass balance calculation, Table 48 shows that the ambient nitrite concentration would increase rapidly during the 24 hr production cycle. Consequently, the nitrite predicted concentrations would certainly produce stress in the salmon and possibly also cause mortalities.

Feed/Day	250										Kg
V = system volume	1000000										L
Q = flow rate through the system	180										L/min
	1 x Fluidised Sand	2 x Sand filters									
Qf = flow rate (1)	2430	1200									L/min
Efficiency (NH4-N) to (NO2-N) (2)	0.9	0.91									
Efficiency (NO2-N) to (NO3-N) (3)	0.6	1.25									
Ammonia Mass Balance	Day 1						Day 2				
t = time	10:00 AM		11:00 AM		10:00 PM		9:00 AM		10:00 AM		min
Ci TAN system	1.51		1.51		1.51		1.51		1.51		mg/L
PR TAN = production rate	5208										mg/min
CR TAN = consuption rate	3302	1649	3300	1648	3294	1645	3293	1644	3293	1644	mg/min
(dCTAN/dt)*V =	-15		-12		-1		0		0		mg/min
CTAN increase	0.00		0.00		0.00		0.00		0.00		mg/L
Cf TAN system	1.51		1.51		1.51		1.51		1.51		mg/L
Nitrite Nitrogen Mass Balance											
Ci NO2-N	0.200		0.252		0.791		1.269		1.310		mg/L
PR NO2-N	3302	1649	3300	1648	3294	1645	3293	1644	3293	1644	mg/min
CR NO2-N	1981	2061	1980	2060	1976	2056	1976	2055	1976	2055	mg/min
(dCNO2/dt)*V	873		863		764		678		670		mg/min
CNO2 system increase	0.052		0.052		0.046		0.041		0.040		mg/L
Cf NO2 system	0.252		0.304		0.837		1.310		1.350		mg/L

(1) Flow rate selected to obtain 20% bed expansion.

(2) (3) Average removal efficiencies worked out by considering data from Table 21 page 115 (samples 7, 8, 9 and 14).

Table 48: Quoys RASs mass balance predicting the trend of ambient ammonia and nitrite concentration during the 24 hr production cycle. The biological trickling filters were replaced with the selected fluidised-sand bed.

2. In both sets of pilot trials (EBCT = 2.3 min.; EBCT = 1.2 min.), backwashing of the pilot filters (water velocity = 34 m/hr) appeared to affect the removal of ammonia and nitrite in the sand filter but did not appear to have any great effect on MnO_2 filter performance.

3. Finally, the sand media did not allow a 50% bed expansion without having to increase significantly the column height and associated pumping head. This was not the case for MnO_2 where a 50% bed expansion was still giving an acceptable column height and pumping head. As a result, the lower bed expansion could negatively affect the nitrite oxidation to nitrate due to an increased risk of anoxic conditions inside the filter bed.

5.3 Financial analysis

Table 49, 50 and 51 compare the estimated capital and operating cost for the fluidised bed biofilters, using manganese dioxide and sand, and the trickling biofilters currently used at the hatchery.

Type of biofilter technology	Fluidised Mn	Fluidised sand	Trickling filter
Capital Cost			
Media cost (£/m^3) [1]	1,200	200	300
Cost of medium (£)	3,750	1,120	21,600
Cost of fiberglass vessel (£) [2]	2,700	3,800	9,000
Cost of assembly (£)	600	600	600
Pump (£) [3]	3,000	3,000	4,500
Pump connection (£)	400	400	500
Eletrical connection for pumps (£)	1,000	1,000	1,500
Total capital cost (£)	**12,650**	**10,120**	**38,000**
Operating Cost			
Electricity (£/y) [4]	1,533	1,971	5,256
Labour (£/y) [5]	1,000	1,000	500
Total operating cost (£/y)	**2,533**	**2,971**	**5,756**

(1) The price for the medias was found from Ferguson Wild Ltd.

(2) The prices for the biofilter vessels were found in literature (Summerfelt and Wade, 1998).

(3) The prices for the pumps were found in a commercial catalogue (Flygt, 2003).

(4) £ 0.05 per Kwhr.

(5) Biofilter maintenance.

Table 49: Comparison of the capital and operating costs related to the installation of the fluidised bed biofilters, using silicate sand and manganese dioxide medias, and trickling biofilters.

Cash flow fluidised Mn				
Year	Capital cost	Operating cost	Cash flow	Cumulative flow
0	12650	0	12650	12650
1	-	2533	2533	15183
2	-	2533	2533	17716
3	-	2533	2533	20249
4	-	2533	2533	22782
5	375*	2533	2908	25690
6	-	2533	2533	28223
7	-	2533	2533	30756
8	-	2533	2533	33289
9	-	2533	2533	35822
10	4775**	2533	7308	43130
11	-	2533	2533	45663
12	-	2533	2533	48196
13	-	2533	2533	50729
14	-	2533	2533	53262
15	375*	2533	2908	56170
NPV @ 5%				
Year	Cash Flow	Discount Factor	Discounted Factor	Cumulative Flow
0	12650	0	0	12650
1	2533	121	2412	15062
2	2533	235	2298	17360
3	2533	345	2188	19548
4	2533	449	2084	21632
5	2908	630	2278	23910
6	2533	643	1890	25800
7	2533	733	1800	27600
8	2533	818	1715	29315
9	2533	900	1633	30948
10	7308	2822	4486	35434
11	2533	1052	1481	36915
12	2533	1123	1410	38325
13	2533	1190	1343	39668
14	2533	1254	1279	40947
15	2908	1315	1218	42165

** Replacement 10% manganese dioxide media every 5 years.*

*** Replacement pump, pump connection and electrical connection.*

Table 50: Cash flow projection (15 years) related to the installation of the fluidised bed biofilter using manganese dioxide media.

Cash flow fluidised sand				
Year	Capital cost	Operating cost	Cash flow	Cumulative flow
0	10120	0	10120	10120
1	-	2971	2971	13091
2	-	2971	2971	16062
3	-	2971	2971	19033
4	-	2971	2971	22004
5	112*	2971	3083	25087
6	-	2971	2971	28058
7	-	2971	2971	31029
8	-	2971	2971	34000
9	-	2971	2971	36971
10	4512**	2971	7483	44454
11	-	2971	2971	47425
12	-	2971	2971	50396
13	-	2971	2971	53367
14	-	2971	2971	56338
15	112*	2971	3083	59421
NPV @ 5%				
Year	Cash Flow	Discount Factor	Discounted Factor	Cumulative Flow
0	10120	0	0	10120
1	2971	141	2830	12950
2	2971	276	2695	15644
3	2971	405	2566	18211
4	2971	527	2444	20655
5	3083	667	2416	23071
6	2971	754	2217	25288
7	2971	860	2111	27399
8	2971	960	2011	29410
9	2971	1056	1915	31325
10	7483	2889	4594	35919
11	2971	1234	1737	37656
12	2971	1317	1654	39310
13	2971	1395	1576	40886
14	2971	1470	1501	42387
15	3083	1600	1483	43870

** Replacement 10% silicate sand media every 5 years.*

*** Replacement pump, pump connection and electrical connection.*

Table 51: Cash flow projection (15 years) related to the installation of the fluidised bed biofilter using silicate sand media.

Cash flow trickling filter				
Year	Capital cost	Operating cost	Cash flow	Cumulative flow
0	38000	0	38000	38000
1	-	5756	5756	43756
2	-	5756	5756	49512
3	-	5756	5756	55268
4	-	5756	5756	61024
5	-	5756	5756	66780
6	-	5756	5756	72536
7	-	5756	5756	78292
8	-	5756	5756	84048
9	-	5756	5756	89804
10	6500*	5756	12256	102060
11	-	5756	5756	107816
12	-	5756	5756	113572
13	-	5756	5756	119328
14	-	5756	5756	125084
15	-	5756	5756	130840
NPV @ 5%				
Year	Cash Flow	Discount Factor	Discounted Factor	Cumulative Flow
0	38000	0	0	38000
1	5756	274	5482	43482
2	5756	535	5221	48703
3	5756	784	4972	53675
4	5756	1021	4735	58410
5	5756	1246	4510	62920
6	5756	1461	4295	67216
7	5756	1665	4091	71306
8	5756	1860	3896	75202
9	5756	2046	3710	78913
10	12256	4732	7524	86437
11	5756	2391	3365	89802
12	5756	2551	3205	93007
13	5756	2703	3053	96060
14	5756	2849	2907	98967
15	5756	2987	2769	101736

* *Replacement pump, pump connection and electrical connection.*

Table 52: Cash flow projection (15 years) related to the installation of trickling biofilter.

Type of biofilter technology	Fluidised Mn	Fluidised sand	Trickling filter
Total (1st year) cost at NPV 5% (£)	15,062	12,950	43,482
Cost per smolt produced [1]	0.02	0.02	0.06
Cost 1 Kg TAN [2]	15	13	44
Cost 1 Kg feed [3]	0.46	0.39	1.32
Total (15 year) cost at NPV 5% (£)	42,165	43,870	101,736
Cost per smolt produced (£)	0.01	0.01	0.03
Cost 1 Kg TAN (£)	9	9	21
Cost 1 Kg feed (£)	0.26	0.27	0.62

(1) 750,000 smolt produced per year.

(2) 2.7 Kg TAN produced per day = 986 Kg TAN produced per year.

(3) 1 Kg feed = 30 g TAN produced.

Table 53: Comparison of the production costs related to the installation of the fluidised bed biofilters, using silicate sand and manganese dioxide medias, and trickling biofilters. The costs are estimated for one year and fifteen years.

Table 51 shows that the trickling biofilter was without doubt the most expensive, which stresses the importance of selecting the right filter configuration particularly when systems with low nutrient levels are considered. The high cost of the trickling biofilter was mainly related to the quantity of plastic-media required and to the operating cost in terms of electricity consumption.

In addition, however, trickling filters contribute to remove carbon dioxide and to provide additional oxygen to the closed system water. Thus, the value of aeration in trickling filter was also considered. Assuming that an average oxygen increase of 1 mg L^{-1} will be found at the outlet of the trickling filter, a total amount of 10 Kg of oxygen per day will be produced by the trickling filter (1 mg L^{-1} x 120 L s^{-1} x 86,400 s). The commercial scale hatchery produced oxygen on site (168 kg/day) using PSA

system with an estimated cost of £ 0.16/Kg O_2 (Owers, Personal communication). Therefore, the trickling filter contributes to oxygen saving of around £ 1.6/day. However, considering the high total cost of trickling biofilter, it would appear that the additional value provided by aeration has limited effect on system production cost.

Regarding the fluidised biofilters it was assumed that the media selected were exploitable for a minimum time of 10 years. As a result, the two biofilters had comparable total costs, but they were differently distributed between the capital and operating costs. The capital cost of the MnO_2 biofilter was slightly higher because of the expensive media, while electricity consumption was greater for the sand biofilter.

Furthermore, the MnO_2 media would appear to be more reliable in oxidising nitrite to nitrate compared with sand. Thus, the value of reliability in MnO_2 media could also be estimated. For example, considering the application of conventional sand media, it can be assumed that nitrite output events will occur with an equal frequency observed during the pilot trials (not including the biological start-up), which is approximately 8 times per year. During these times, Figure 48 shows that a dangerous nitrite concentration can be rapidly establish in the closed system. If an increase of 1% in mortality will happen during each event as a result of nitrite production from the sand media, then the hatchery will have an additional loss of 60,000 fish per year (1% of 750,000 x 8), which at £ 0.80/ fish will cost about £ 48,000. The application of MnO_2 media could prevent these fish losses by providing a more reliable oxidation of nitrite to nitrate compared with sand media.

Chapter 6
Discussion and conclusion

It was clear from the beginning of this work that the investigation of MnO_2 as a supporting media for bio-filter would be complex. Physical, chemical and biological factors, singularly and/or in combination, could affect factors such as manganese solubility and the performance of MnO_2 in terms of metabolite removal and external impact. For this reason a set of experiments under laboratory conditions, in the absence of biological activity, was initially carried out to investigate aspects relating to the surface area and morphology of different types of commercial bio-media including MnO_2. The outcomes of these studies were then used to compare ammonia and nitrite adsorption and reactions on MnO_2 against those on silicate sand media. The laboratory results were then scaled up at a level of field trials based on a commercial fish farm, which compared the metabolite removal performance of silicate sand and pure MnO_2 media in systems that are representative of the biological activity found in a bio-filter. As a final stage, based on these results, and on typical production and risk scenarios, the cost-effectiveness of manganese systems in aquaculture was compared with that of alternatives.

6.1 Laboratory studies

6.1.1 Specific surface area and surface morphology

The manganese dioxide specific surface area measured in these trials by BET adsorption methods on commercial ore samples used was lower than values found in the literature. Balistrieri and Murray (1982) reported a value of 74 ± 1 $m^2 g^{-1}$ on δ-MnO_2[1] samples passed through a fine (400 μm) sieve, compared with values of 14.25 $m^2 g^{-1}$ found here for commercial sample of β-MnO_2[2] crushed ore of BS 16/30 grade (1.19 mm – 0.595 mm). A commercial β-MnO_2 ore with a different grade size (0.6 – 0.5 mm) was also tested, which produced a similar BET value to that of the BS 16/30 grade β-MnO_2 ore. This may suggest that the internal surface area of commercial β-MnO_2 is much greater than the external surface area and so, to a certain extent; the specific surface area appears to be independent of particle size. If this is true, then MnO_2 might have an additional internal surface area available to bacteria. Assuming that this internal surface area may be effective, then using MnO_2 media could permit a reduced bio-filter size and consequently reduce its costs.

However, Balistrieri and Murray (1982) only gave an upper grade size of the sample used in the BET measurements. While this is comparable to the grade size used here, no minimum size was recorded. However, in their trials the δ-MnO_2 was produced by chemical precipitation and "colloidal suspensions" of δ-MnO_2 were reported to be

[1] *The delta phase, prominently represented by the mineral birnessite.*

[2] *The beta phase, represented by the mineral pyrolusite.*

formed. This implies a much smaller grain size than the 0.595 mm minimums employed in the present trials. On this basis it is not possible to exclude differences in grain size as the origin of the variation of surface area values obtained in this study and elsewhere.

However, several researchers (Yates, 1975; Pyman and Posner, 1978) have reported problems in determining and interpreting surface area measurements of amorphous oxides. The discrepancy between reported and measured surface area could also be explained by differences in chemical composition and degree of crystallinity between MnO_2 samples. Commercial samples, characterized as pyrolusite (β-MnO_2) with high degree of crystallinity (Di Ruggiero, 1989; Rusin *et al.*, 1991), have a manganese content of 70 – 75% as MnO_2 (Bermingham, 2001), while the δ-MnO_2 form seems to have a poor degree of crystallinity and, if prepared by chemical precipitation, should be of much higher purity. Given these variables the variation in surface area observed is probably not significant.

By combining specific surface area data with surface morphology observations it may be possible to determine more realistically the suitability of a medium for sustaining a bacterial population. As expected, the present BET studies revealed that GAC has a significantly greater surface area per particle than MnO_2 (approximately 50 times as great), which in turn has a significantly greater surface area per particle than SS, VR and SG (approximately 10 times as great). In addition, the micrograph observations (Macro x 320) of GAC suggested that the complex surface morphology with a higher numbers of creeks ($\geq$ 1000 μm^2) and cavities ($\geq$ 500 μm^2) should be capable of

supporting a superior number of nitrifying bacteria (≈ 2 μm) compared with SS, VR, SG and MnO_2.

However, the surfaces of silicate sand and MnO_2 showed a complex microstructure with numerous pores (≤ 500 μm^2), able to provide a higher surface area to the attachment of single bacterial cells than the GAC surface. Therefore, the high internal surface area of GAC, while suitable for bacterial colonisation, may not be effectively utilised because of reduced access due to bio-fouling and limited water flow to provide oxygen and nutrients requirements. Therefore, a greater number of nitrifying bacteria could be associated with the surface of MnO_2 despite its smaller surface area (as measured by BET). Consequently, the MnO_2 medium could actually have a higher capacity to oxidise ammonia and nitrite compared with the GAC medium. However, no published studies have been found that specially examine the MnO_2 relationship between internal and external surface area and whether this could be affected by the way in which the commercial ore is processed.

6.1.2 Manganese solubility

In chapter 5, it was suggested that partial replacement of the sand media with manganese dioxide (20% by volume) would increase the nitrite removal rate from the existing rapid sand filters in commercial application. While the removal of nitrite would result in a significant reduction in risk to aquatic stocks, the presence of dissolved manganese itself, and the influence of operating conditions on this, might also be important. Since manganese is potentially toxic to aquatic life, some knowledge of its solubility was required.

As expected, equilibration experiments in distilled water showed that the pH was a primary factor in determining MnO_2 solubility. At an inlet pH of 5.7, $[Mn_{sol}]$ increases with a slow increase with time even after 20 minutes when, under these experimental conditions, it reaches a value of 17.5 $\mu g\ L^{-1}$. These results were in general agreement with Bermingham (2001), who showed an inverse relationship between pH and MnO_2 solubility with a marked increase in $[Mn_{sol}]$ observed at pH 5 and below. During Bermingham's experiments, $[Mn_{sol}]$ was generally below 50 $\mu g\ L^{-1}$ as long as the pH was maintained below 5.

Exley and Phillips (1988) have reported acute effects of soluble manganese at > 770 $\mu g\ L^{-1}$ and sub lethal gill damage at 100 – 500 $\mu g\ L^{-1}$. Thus, $[Mn_{sol}]$ levels observed experimentally are well below the indicated toxicity levels. However, considering that relatively small pH fluctuations within few days (± 0.5) can occur in RAS (Owers personal communication) and that an exponential increase in $[Mn_{sol}]$ was observed around pH 5 (Bermingham, 2001), the catalytic MnO_2 filters could became extremely dangerous in an aquaculture context if the pH of the system is not set with some margin of security, *i.e.* pH > 6.

6.1.3 Chemical action of silicate sand and manganese dioxide on ammonia and nitrite

The concentration of ammonia and nitrite used during the laboratory trials (5 and 10 mg L^{-1}) were higher compared with the concentration of these metabolites found in typical aquaculture conditions; ranging from very low ($\leq$ 0.1 mg L^{-1}) up to few mg (1-3 mg L^{-1}). This difference was particularly true in the case of nitrite, which is usually low (0.1- 1 mg L^{-1}) in aquaculture conditions. However, higher ammonia and nitrite concentration were used during the laboratory trials to increase the probability of highlighting chemical interactions between media and the metabolites.

Considering that the surface of silicate sand is negatively charged and that the surface charge of MnO_2 is negative in the pH range found in aquaculture, it was initially expected that NH_4^+ would most likely interact more strongly with both media by electrostatic attraction than NO_2^- would. The results of the batch trials indicated that NH_4^+ was strongly adsorbed by both sand and MnO_2. However, despite the implied electrostatic repulsion, the NO_2^- was also strongly adsorbed by the MnO_2 medium with an adsorption rate, which was over four times as great as that for NH_4^+.

The batch trial, using double distilled water also showed that NH_4^+ is only slowly converted to NO_3^- even with a prolonged contact time, while in the presence of MnO_2, NO_2^- is rapidly oxidised to NO_3^-. However, when pilot trials were carried out with reconstituted soft and hard fresh water, the chemical action of MnO_2 on NO_2^- was strongly reduced, resulting in minimal differences from the silicate sand medium. In this case, the presence of divalent cations in solution, such as $Ca^{2+}$and Mg^{2+}, probably

enhanced electrostatic attraction for anion such as NO_2^-, but the surface chemistry of MnO_2 changed as a consequence and the adsorption and/or oxidation of NO_2^- to NO_3^- is inhibited.

Since the chemical interactions between the media and the metabolites appeared to be negligible under these conditions, the degree of metabolite removal by sand and MnO_2 filters was therefore expected to be mainly a function of the interactions between the microbial growth/activities and the media within a specific biological community established inside the filter beds. Here also, however, there may be specific physico-chemical interactions which may enhance the performance of biological oxidation compared with sand filters.

6.2 Field studies

Due to the limited resources available for the investigation on MnO_2 as supporting media in bio-filter, only two full-scale pilot filters could be installed in the commercial hatchery to compare the metabolite removal performance of silicate sand and manganese dioxide ore. These were operated in pressurised modes, and performance also compared with the trickling filter system used in normal hatchery operation. Ideally, three or more filter replicates per each type of media should be used to compare the biological process and obtain statistically significant results. However, to compensate to some degree for the lack of filter replicates, larger numbers of measurements in different conditions were recorded during the 6 months trial. After biological start-up, tests were also carried out to investigate the effect of

backwashing the filters, and the effect of contact time, on metabolite removal performance.

6.2.1 Biological start-up

The four weeks required for the two pilot filters to complete the biological start-up was in broad agreement with data found in literature (Lawson, 1995). The sand and MnO_2 pressurised filters behaved similarly during the biological start-up period; ammonia oxidation was almost completed in three weeks. However, a net production of nitrite occurred during this period, stopping at the end of week 4, suggesting the beginning of the biological start up with respect to nitrite removal. This is also consistent with literature, where as a result of an initial shortage of nitrite, a delay of 10 –14 days period was observed for the development of nitrite bacteria compared to ammonia bacteria (Lawson, 1995).

In pure water, the batch trials of the MnO_2 showed superior sorptive and reaction properties for metabolites compared with silicate sand, but this enhanced performance rapidly disappeared when other chemical species were added in solution to produce reconstituted fresh water. This effect was confirmed in the pilot trials, in that the Mn and sand filters experienced the same start up characteristics. If the MnO_2 filter had maintained sorptive properties during the field experiment, favouring nitrifying bacterial colonization by providing a constant substrate to feed on, then a shorter start-up might have been expected compared to the sand filter.

To complicate the study further, the laboratory experiments did not consider the interaction between humic acids and MnO_2, which is also able to adsorb organic substances, affecting its interactions with other chemical species. This may also possibly favour heterotrophic bacteria growth, the main competitors of nitrifying bacteria. Furthermore, as soon as a biofilm forms on the surface of the MnO_2 media, it is likely to provide a physical barrier to the physico-chemical interaction of metabolite and organic substances, and so the theoretical sorptive and reaction properties of MnO_2 could be modified.

Notable concentrations of organic materials (BOD = 10.20 mg L^{-1}) were measured during the biological start-up. Tipping and Heaton (1983) study, based on adsorption of humic substances by manganese oxides, suggests that organic materials encountered here at the commercial hatchery may be partially adsorbed on the surface of MnO_2 media. Moreover, the water harness measured during the field pilot trials was 100 mg L^{-1} (as $CaCO_3$), thus, a higher content of Ca^{2+} and Mg^{2+} was found compared with the reconstituted soft water used during the lab pilot trials.
Overall, this evidence suggests that the presence of other inorganic and organic chemical species would almost certainly inhibit the chemical potency of MnO_2 on metabolites and in particular nitrite oxidation. Nevertheless, because much lower metabolite concentrations were tested during the field experiment (about 100 times less) compared with the trials carried out in the laboratory, it is possible that any differential chemical interaction occurring inside the MnO_2 filter bed was too small to be accurately measured by the analytical techniques for ammonia and nitrite. As only a limited number of samples were collected during the biological start up, the

statistical significance of the two sets of data collected during the pilot filter experiment is further limited.

In hindsight, it would have been interesting to compare the oxygen consumption between the two filters during the biological start-up. This may have helped highlight diversity in the biological community established inside the filters. In particular, knowing the amount of oxygen used by nitrifying bacteria, it would be possible to compare the oxygen consumption used by the heterotrophic community and therefore estimate the potency of the two bacterial communities within the two filters. Unfortunately, no oxygen measurements were carried out during this stage of the trials to verify this hypothesis. Considering that there were notable concentrations of degradable carbon at the inlet streams, then the main difference would primarily be due to the ability of MnO_2 to pick-up the poorly-degradable C, *i.e.* the humic acids.

6.2.2 The effect of filter backwashing

Backwashing is required to remove the clogging deposits trapped inside the filter bed. Generally, the smaller the diameter of the filter media, the faster the filter bed will block, requiring more frequent backwashing. Typically, in the aquaculture sector, granular media bio-filter designs, that require backwashing, are regularly cleaned every 24 – 48 hours. The backwashing cycle can be carried out with water or alternatively with combined air and water. The latter gives better cleaning because it maximizes particle collisions and abrasion (Ahmad *et al.*, 1998). However, because of the design adopted for the field pilot trials, the filter media was cleaned using only a water backwashing.

When start up was completed, in order to study the effect of backwashing on filter performance with respect to bacterial population, additional measurements were carried out 2, 4, 6 and 24 hours after backwashing to compare ammonia and nitrite removal efficiency and removal rate of the sand and MnO_2 pilot filters. The following observations were apparent:

1. From the measurements carried out 24 hrs after filter backwashing, the average ammonia removal efficiency was comparable in the two pilot filters, but the MnO_2 pilot filter showed significantly higher nitrite removal efficiency;
2. From measurements made 2 hours afterwards, backwashing the filters appeared to seriously affect the removal efficiency of ammonia and nitrite in the case of the sand filter, but did not appear to have any great effect on MnO_2 filter performance;
3. From the measurements carried out 4 and 6 hours after filter backwashing, the sand filter shows improved ammonia and nitrite removal efficiency with time, while the MnO_2 filter showed no time-dependent removal efficiency over this time period;

The filter flow rate was adjusted to obtain a water velocity inside the filter beds of about 5 m/hr under working conditions. This is comparable to the average velocity found in typical rapid sand filters (Franklin and Pilkingston, 2001). However, this water velocity was much lower compared with fluidised-bed technology, which have an average water velocity of 50-100 m/hr (Timmons and Summerfelt, 1998). During filter backwashing, the water velocity applied to the medium increased considerably

from this value. Because of the lower density of sand compared with MnO_2 the backwashing flow rate for the manganese medium (34 m/hr) was higher than for sand (22 m/hr); at these rates the same level of bed fluidisation was maintained in both filters. In this way clogging deposits within the filter beds were removed, but this probably also reduced the nitrifying autotrophic bacteria biomass by partially removing bacterial cell and bacteria flocks attached to the surface of the media and/ or to solid particles trapped in the filter bed (Golz *et al.*, 1999).

Nevertheless, assuming the MnO_2 media provides a better 'anchor' for bacterial cells compared to silicate sand, it may be able to hold a higher number of bacterial cells on its surface at the end of the backwashing, thus maintaining a higher capacity to oxidise ammonia and nitrite. This explanation of improved capacity is further supported by the fact that the water velocity applied to the MnO_2 filter during backwashing was considerably greater then for the sand filter. Under these conditions, the scouring of the MnO_2 particles should be greater and a lower oxidation capacity would be expected for the MnO_2 media. It would have been a good idea to compare the amounts and maybe particle sizes of the solids flushed out of each filter. Undoubtedly, this would have helped to establish if MnO_2 media actually provides a better 'anchor' for bacterial cells compared to silicate sand.

6 hours after the end of the filter backwashing the sand filter fully recovered its properties showing an ammonia removal rate similar to that observed 24 hours after filter backwashing. In particular, the ammonia removal efficiency 24 hrs after the end of filter backwashing was about 90% and dropped to 80% as a result of backwashing (2 hours after backwashing). The ammonia removal efficiency fully recovered to 90%

6 hrs after the end of filter backwashing. Thus, in broad term, a doubling time for ammonia oxidizer bacteria of about 60 hours was found for the sand filter. This was considerably higher compared to the doubling time for nitrifying bacteria of 7 – 8 hours found by Watson (1971) and Bock *et al.* (1989) under ideal conditions and 26 hours found by Shilo and Rimon (1982) in fishponds. This difference may be primarily related to temperature; the average temperature was 14 °C during the pilot trial while temperature above 20 °C was observed in the above research.

In contrast to the sand filter, the MnO_2 pilot filter showed significantly higher nitrite removal efficiency 24 hours after filter backwashing. This may be explained by taking into account that the doubling time for nitrite oxidizing bacteria appears to be longer than that of ammonia oxidizers. For example, Shilo and Rimon (1982) measured a doubling time of 26 h for ammonia oxidizer and 60 h for nitrite oxidizer nitrifiers present in fishponds. This is also in agreement with the reaction stoichiometry of *Nitrosomonas* and *Nitrobacter*, where the ammonia oxidizer has a higher specific yield than the nitrite oxidizer.

Therefore, it is possible that by applying filter backwashing every 24 hrs, as might be practiced in a typical production system, the nitrite oxidizer production rate in the sand filter would not be sufficient to replace the number of bacterial cells washed away during filter backwashing. However, in the case of the ammonia oxidizers a greater number of bacteria cells are produced during the 24 hrs filter cycle allowing replacement of those cells lost during backwashing and so, with respect to ammonia removal, a similar performance to the MnO_2 filter was obtained. Furthermore, the backwashing can remove the external bacteria layers and revive the internal bacteria

layers, which then can be exposed to higher metabolites and O_2 levels. This could effect the nitrite removal more than the ammonia removal, since nitrite oxidisers are more sensitive to environmental stresses than ammonia oxidisers (Lawson, 1995).

Conventional bio-filters hold a diverse biological community generally composed of nitrifying bacteria and heterotrophic bacteria. The heterotrophic organisms typically grow at significantly higher rates than autotrophic bacteria. Thus, where carbon source are available for heterotrophic bacteria, nitrifying biofilms can be heavily overgrown by heterotrophs, which usually dominate the biofilm or bio-particle surface and utilize oxygen before it can reach the nitrifying bacteria. However, with manganese dioxide used as a supporting media in bio-filter, it may be that a different biological community could develop compared with conventional bio-filter. For example, because of manganese levels and iron impurities, manganese dioxide media could enhance the development of the iron bacterial community within the filter bed, competing with nitrifying bacteria and heterotrophic bacteria for surface space and oxygen up-take.

From measurements carried out 24 and 2 hours after backwashing the filters, increased ammonia and oxygen removal was found for increased inlet ammonia concentrations suggesting that the inlet oxygen concentrations was mainly used by nitrifiers to oxidize ammonia to nitrate. This would be supported by the observation of the low content of suspended solids and BOD_5 in the raw water.

These conditions would probably not favour heterotrophic bacterial colonization, by depriving them of a constant substrate to feed on. Nevertheless, oxygen removal was

found to exceed the theoretical oxygen requirements for ammonia removal in both pilot filters, suggesting that a modest and similar degree of heterotrophic activity was present in both filters. It would have been a good idea to compare the amounts of carbon entering these systems and the levels of removal, and relate them to the oxygen consumption. This would have probably helped to give a better picture of the biological community established inside the filters.

Furthermore, the difference between theoretical (nitrifying bacterial O_2 usage) and observed oxygen removal decreased by a comparable amount after backwashing, suggesting that similar bacterial communities were possibly established inside the two filters. It may be that the manganese dioxide media was able to provide an alternative substrate for the establishment of an iron bacteria community (Buzio and Alberti, 1995). However, by looking at the existing evidence, iron bacteria did not compete for oxygen uptake with the dominant nitrifying bacteria.

6.2.3 Effect of reduced contact time

Various researchers suggest that for nitrification, retention time is the primary factor affecting metabolite removal (Liao and Mayo, 1974; Muir, 1982; Timmons and Losordo, 1994). This appears to be in agreement with the pilot trial results. As a result of the reduced contact time, both media showed a drop in ammonia and nitrite removal efficiency, but higher ammonia removal rates, confirming that retention time was affecting removal.

With respect to backwashing, measurements carried out 2 hrs and 24 hrs after filter backwashing, showed that MnO_2 has a significantly higher ammonia and nitrite removal efficiency compared to silicate sand. In particular, comparing results obtained with double the contact time, the silicate sand pilot filter showed significantly lower nitrite removal efficiency and produced a continuous output of nitrite. From these results the sand media appeared to be incompatible for this application in RAS because of the high accumulation of nitrite produced, where on average about a third of the ammonia oxidised was only converted to nitrite.

The accumulation of nitrite in RAS is often attributed to environmental factors; in particular conditions of low oxygen concentration, where oxygen levels limit nitrite oxidizers more than ammonia oxidizers (van Rijn and Rivera, 1990). However, during this investigation, environmental factors, including oxygen concentrations, should not have caused nitrite accumulation in the pilot sand filter. This suggests that the nitrite output is probably caused by a lower nitrite removal rate compared to that for ammonia oxidation. Furthermore, there is the possibility that backwashing can remove nitrite oxidisers and further increase nitrite accumulation. The lower nitrite removal rate on silicate sand media could be reinforced if it presents a poorer 'anchor' for bacterial cells compared to MnO_2.

Moreover, nitrite accumulation in the pilot sand filter could also be explained by a substrate limited diffusion process (Nijhof and Klapwijk, 1995). In contrast, the MnO_2 medium's adsorption properties could reduce the partial outward diffusion of nitrite (Bovendeur, 1989), favouring the complete oxidation of ammonia to nitrate without

need to achieve an equilibrium condition between the bulk fluid and the upper biofilm layer.

Overall, mean TAN removal rates expressed as g TAN/day-(m^3 of filtration media) varied from about 531 to 798 for the MnO_2 filter, 511 to 669 for the sand filter. These results are in agreement with Tsukuda *et al.* (1997), who investigated the ammonia removal from silicate sand in cold water (15 °C). At comparable grade size the mean TAN removal rate was 510 for the clean static bed and 350 for the expanded bed. In contrast, Westerman *et al.* (1993) found that the mean TAN removal rates varied from about 150 to 230 for upflow sand filters, 250 to 290 for fluidised filters. These filters were tested in warm water, at mean temperature of 27-28 °C, and mean suspended solids level that varied from 10 to 30 mg L^{-1}. Thus, this difference may be primarily related to suspended solids; the mean suspended solid level was 1 - 2 mg L^{-1} during the pilot trial. It may be that the high level of organic carbon favoured the proliferation and competition of heterotrophic against the nitrifying bacteria.

6.2.4 Manganese solubility

In a pH range between 6.79 and 7.40, an output of $[Mn_{tot}]$ from the outlet of the MnO_2 filter was not detected from samples collected during the entire filter trials. However, one of the main concerns about the application of MnO_2 in the RAS context was the interaction between manganese and organic compounds, where manganese dissolution may result from such interactions. Furthermore, a variety of chemoheterotrophic and chemoautotrophic bacteria could potentially affect the

solubility of solid MnO_2, particularly in anaerobic conditions, encouraging microorganisms that could utilize MnO_2 as an alternative electron acceptor.

During these trials, it appears that the filter environment was maintained in continuous aerobic conditions because frequent backwashing and stirring by hand produced relatively high concentrations of oxygen, as detected at the outlet of the MnO_2 filter. However, on the surface of the MnO_2 medium two different biofilm zones may exist, including an 'active thickness' and an 'inactive thickness' (Kornegay and Andrews, 1968; LaMotta, 1976). In the 'inactive thickness', the inner layer closer to the support media interface, the diffusion of nutrients would become a limiting factor, thus an anaerobic environment might be established near the surface of the MnO_2 media.

Although the pilot filters were fed with raw water having low BOD_5 and TSS, the small diameter of the filter media would promote the accumulation of organic compounds inside the MnO_2 filter bed. Thus, the fact that no significant difference on output of $[Mn_{tot}]$ was detected from the outlet of the Mn filter, between 2 hr and 24 hr after filter backwashing, would suggest that the building up of suspended solids trapped inside the filter bed was insufficient to solubilize MnO_2. Similarly, the presence of nitrifying bacteria and heterotrophic bacteria did not appear to affect the solubility of solid MnO_2. Nevertheless, it may be that the manganese reduced by organic compounds and/or by bacteria activities would be re-adsorbed on the surface of the solid MnO_2 and possibly re-oxidised to Mn III-Mn IV. Overall, the manganese system can be considered safe in the normal pH range found in aquaculture conditions. However, the filter bed must be kept in clean and constant aerobic

conditions, avoiding the processes of clogging and channelling of water through the media. In this environment, even if a small amount of Mn is solubilised, it will be re-oxidised on the surface of the MnO_2.

6.3 Technical analysis

The observations carried out in the commercial hatchery showed that while the ammonia in the system was controlled, it was difficult to predict the levels of nitrite. This was because the main biological treatment process, the trickling filter units, had a continuous net production of nitrite.

Thus, two fluidised-bed biofilter columns had been designed. One of the columns used sand as the carrying media, and the other used MnO_2. These two biological filters were designed to have the same ammonia removal capacity as the existing trickling filters, but were also intended to be able to complete the ammonia oxidation to nitrate in order to control the nitrite concentrations.

The MnO_2 is of a higher density than sand, requiring a higher water velocity and flow rate compared to sand media. However, due to the higher ammonia and nitrite removal rates, a shorter retention time and a smaller volume of media were required. Thus, from a design point of view, the MnO_2 as the carrying media would offer the following advantages compared with sand media:

- Smaller and more compact
- Lower total pumping head and power required

- Much easier to size, to ensure safety on nitrite removal

It seems difficult to predict and control nitrite levels in RAS because the nitrite removal capacity in nitrifying systems is variable and sensitive to environmental disturbance (Otte and Rosenthal, 1979; Poxton *et al.*, 1981; Bovendeur *et al.*, 1987; van Rijn and Riviera, 1990; Kamstra *et al.*, 1998). As observed in the pilot trials, when the oxidation of ammonia is incomplete, a higher concentration of nitrite can occur, which can even impose limitations on the system feed input and thus on RAS production.

The value of the MnO_2 system was assessed for the commercial salmon hatchery. Under these conditions, although silicate sand and MnO_2 fluidised biofilters have comparable total operating costs inclusive of capital and maintenance, the overall economy could be in favour of the MnO_2 media because it is much easier to size to ensure safety on nitrite removal.

Currently the trend is towards big RASs, for example Wilton (2002) presented a design for a 50 tonnes (2 crop per year) salmon hatchery operation approximately equivalent to 1 million smolts per year. Assuming that the feed rate is 2% per day, at the peak of biomass (25 tonnes) the feed input in the RAS will be about 500 kg/day. During the design study, 0.08 m^3 of biofilter / kg feed was required for the MnO_2 filter and 0.12 for the sand filter. Thus, the required bio-filter volume for 500 kg feed/day will be around 40 m^3 for MnO_2 and 60 m^3 for sand. Because of the high biofilter volume required, the fluidised bed would ideally be designed as a number of smaller units, which will also offer the advantage of flexibility of operation. As the MnO_2 is

much easier to size, a smaller number of biofilter units are expected to be needed compared to the sand filter. Thus, as a broad estimate, 4 biofilter units could be used for the MnO_2 and 6 units for the sand. Hence, the application of MnO_2 technology in big RASs could allow an extra saving in capital (fibreglass vessel, pump, etc.) and operating cost (labour for filter maintenance) compared with the study carried out in these trials.

A biofilter volume of 1.2 m^3/kg feed was required for the trickling filter compared to 0.08 m^3/kg for the MnO_2 filter and 0.12 m^3/kg for the sand filter. Thus, for the 50 tonnes hatchery presented by Wilton (2002), the required filter volume will be around 600 m^3 for the trickling filter compared to 40 m^3 for the MnO_2 filter and 60 m^3 for the sand filter. This suggests that for a large RAS operating at typically low nutrient levels, a trickle filter is for reasons of size and hence management complexity, much less likely to be practical option. However its role in aerating the process water, and possibly striping off CO_2 would have to be considered, and if necessary, alternative treatment employed.

The pilot trials showed that MnO_2 has a superior reliability in completing ammonia oxidation to nitrate compared with the sand filter. Therefore, as a result of using MnO_2, the mortality risk was estimated to be lower compared to the sand filter, with a total reduction in fish loss per year of approximately 10%, or some £65,000 based on an estimated biomass value of £650,000. By comparison with any marginal costs of using MnO_2 this suggests that a small investment in improving reliability would be well compensated. Were consequential losses also to be considered, this impact, and hence the value of investing in a MnO_2 system would be even greater.

6.4 Conclusions

From the pilot trials results, the superior performance of MnO_2 media compared with sand appears to be mainly related to the physical structure of the manganese ore. Nevertheless, a situation may exist where by in simply using pilot filters inlet and outlet measurements it is difficult to highlight organic species and bacterial interaction with MnO_2 substrate, and their ability to solubilize it. Thus, the soluble manganese could be re-adsorbed and oxidized inside the filter bed by the chemical equilibria associated with MnO_2 / Mn^{2+} and / or by biological oxidation.

To investigate the effects on biofilm and media physical structure, the sand and MnO_2 pilot biofilters should be left running for a longer period of time (at least one year). In particular, the dissolution of MnO_2 ore could increase the size of the internal surface available to bacteria and constantly regenerate the MnO_2 ore / biofilm interface contributing to reducing the 'inactive thickness' biofilm zone. A comparison on the evolution of efficiency with time could also be studied.

Furthermore, the pilot trials were carried out in optimal environment conditions for nitrifying bacteria. The oxygen levels inside the filter beds were high, while the levels of organic substances were low. It would be interesting to compare the removal of metabolites and manganese solubility for sand and MnO_2 media in conditions of low oxygen and high levels of organic substances, thus, investigating MnO_2 media in conditions where heterotrophic bacteria overcome autotrophic bacteria.

To investigate the subject further, it would be interesting to study the microenvironment at the MnO_2 media-biofilm interface. It would useful to characterise the number and types of bacteria attached to the media and to examine the manganese solubility as a function of biofilm thickness. Facultative bacteria may be able to use MnO_2 media as the terminal electron acceptor if an anaerobic environment is established near the surface of the media. This could drastically increase the level of soluble Mn, which would not be re-oxidised on the surface of the MnO_2.

Based on the results obtained, even if further investigation is required on MnO_2 as a supporting media in biofilter, a comparison between the use of silicate sand and MnO_2 can be set out, as follows:

The disadvantages of using MnO_2:

- At pH below 6, the media could became soluble and toxic to fish
- The media is more expensive to buy: £ 1,200/m^3 for MnO_2 against £ 200/m^3 for silicate sand

The advantages of using MnO_2:

- As it has a higher metabolite removal efficiency, the design of the column is easier because it requires a lower volume of media
- The media is more reliable in converting ammonia to nitrate without producing residual output of nitrite

Manganese system has a comparable total cost to conventional sand media, but using the Mn technology provides a more reliable control of nitrite in RAS. A broad risk assessment was carried out showing that Mn could cut 10 % of fish mortality per year compared to sand media, thus, reducing the RAS production cost. However, depending on a wide range of environments, RAS design and species targeted, this saving has to be balanced against the potential risk of manganese toxicity.

Furthermore, Mn could just be integrated in RAS as a complementary treatment for the removal of nitrite, in conditions where the main biological treatment is periodically unstable and not able to complete the oxidation of ammonia to nitrate. Thus, further studies need to be carried out to determine design and operational requirements for practical applications. For example, considering that Mn has a high density, pressurized filter technology could provide a more cost effective alternative to a fluidised-bed design, particularly if used as complementary system to improve existing biological treatment.

References

Ahmad, R., Amirtharajah, A., Huck, P. M. (1998). Effects of backwashing on biological filters. *J. Am. Wat. Wks. Assoc.*, **90**, (12), 62.

Alleman, J. E. (1985). Elevated nitrate occurrence in biological wastewater treatment systems. Water Sci. and Technol., **17**, 409-19.

American Public Health Association. (1989). Standard Methods for the Examination of Water and Wastewater. 17th ed., Washington, DC.

Anderson, J. H. (1964). Studies on the oxidation of ammonia to hydroxylamine by *Nitrosomonas*. *Biochem. J.*, **92**, 1C-3C.

Anthonisen, A. C., Loehr, R. C., Prakasan, T. B. S., Srinath, E. G. (1976). Inhibition of nitrification by ammonia and nitrous acid. *J. Wat Poluut. Control Fed.* **48**, 835-852.

Antonie, R. L. (1976). Fixed biological surfaces-wastewater treatment: the rotating biological contactor. CRC Press Inc., Cleveland Ohio. 200pp In: Timmons, M. B., Losordo, T. (1994). Operating and Managing Water Reuse Systems. Engineering Design and Management. Elsevier: Developments in Aquaculture and Fisheries Science, Vol. 27.

Archibald, F. S., Fridovich, I. (1981). Manganese and defences against oxygen toxicity in *Lactobacillus plantarum*. *J. Bacteriol.*, **145**, 442-451. In: Gounot, A. M. (1994). Microbial oxidation and reduction of manganese: Consequences in groundwater and applications. *Microbiology Reviews*, **14**, 339-350.

Audic, J. M., Faup, G. M., Navarro, J. M. (1984). Specific activity of *Nitrobacter* through attachment on granular media. *Wat. Res.*, **18**, 745-750.

Balistrieri L. S., Murray J. W. (1982). The surface chemistry of δ-**MnO2** in major ion seawater. *Geochimica et Cosmochimica Acta*, **46**, 1041-1052.

Bartlett, R. J. (1981). Nonmicrobial nitrite-to-nitrate transformation in soils. *Soil Sci. Soc. Am. J.*, **45**, 1054-1058.

Bath, R. N., Eddy, F. B. (1980). Transport of nitrite across fish gills. *Journal of experimental zoology*, **214**, 119-121.

Bazin, M. J., Cox, D. J., Scott, R. I. (1982). Nitrification in a column reactor: limitations, transient behavior and effect of growth on a solid substrate. *Soil Biol. Biochem.*, **14**, 477-487.

Beckman, G., Snapp, G., McCarthy. T. (2002). Design of a 100 Ton Per Annum flatfish farm. *International Conference on Recirculating Aquaculture*-Virginia Tech - AES Section – Commercial Recirculating Systems, pp.289.

Bermingham, M. (2001). The solubility of manganese dioxide ore as a function of pH and solution type. B.Sc. (Hons) Thesis, University of Stirling.

Blancheton, J. P. (2000). Developments in recirculation systems for Mediterranean fish species. *Aquacultural Engineering*, **22**, 17-31.

Boch, E., Koops, H. P., Harms, H. (1989). Nitrifying bacteria. In: Schlegel, H. G., Bowien, B. (Eds.), Autotrophic Bacteria. Science Tech, Madison, WI, pp. 81-96.

Boch, E., Koops, H. P., Harms, H., Ahlers, B. (1991). The biochemistry of nitrifying organisms. In: Shively, J. M., Barton, L. L. (Eds.), Variations in Autotrophic Life. Academic Press, London, pp. 171-200.

Bovendeur, J., Eding, E. H., Henken, A. M. (1987). Design and performance of a water recirculation system for high-density culture of the African catfish, *Clarias gariepinus* (Burchell 1822). *Aquaculture*, **63**, 329-53.

Bovendeur, J. (1989). Fixed-biofilm reactors applied to waste water treatment and aquacultural water recirculating system. Ph.D. thesis, Agricultural University Wageningen, The Netherlands. In: Nijhof, M., Klapwijk, A. (1995). Diffusional transport mechanisms and biofilm nitrification characteristics influencing nitrite levels in nitrifying trickling filter effluents. *Wat. Res.*, Vol. **29**, No. 10, pp. 2287-2292.

Bovender, J., Zwaga, A. B., Lobee, B. G. J., Blom, J. H. (1990). Fixed-biofilm reactors in aquacultural water recycle systems: effect of organic matter on nitrification kinetics. *Water Res.*, **24**, 207-213.

Brunauer, S., Emmett, P., H., Teller, E. (1938). Adsorption of gases in multimolecolar layers. *J. Amer. Chem. Soc.*, **60**, 309-319. In: Balistrieri L. S., Murray J. W. (1982). The surface chemistry of δ-**MnO2** in major ion seawater. *Geochimica et Cosmochimica Acta*, **46**, 1041-1052

Burns, R. G. (1976). The uptake of cobalt into ferromanganese nodules, soils, and synthetic manganese (IV) oxides. *Geochim. Cosmochim. Acta*, 40, 95-102. In: Balistrieri L. S., Murray J. W. (1982). The surface chemistry of δ-**MnO2** in major ion seawater. *Geochimica et Cosmochimica Acta*, **46**, 1041-1052.

Burdige, D. J., Nealson, K. H. (1986). Chemical and micro-biological studies of sulphide-mediated manganese reduction. *Geomicrobiol. J.*, **4**, 361-387.

Burdige, D. J., Dhakar, S. P., Nealson, K. H. (1992). Effects of manganese oxide mineralogy on microbial and chemical manganese reduction. *Geomicrobiol. J.*, **10**, 27-48.

Buzio, S., Alberti, S. (1995). Esperienze su differenti mezzi filtranti utilizzati per l'eliminazione del manganese dalle acque sotterranee. Dipartimento di Scienze della Terra, Universita degli studi di Torino, 102B-123B.

Characklis, W. G. (1981). Fouling biofilm development: a process analysis. *Biotech. Bioengng*, **23**, 1923-1960.

Characklis, W. G., Trulear, M. G., Bryers, J. D., Zelver, N. (1982). Dynamics of biofilms processes: methods. *Wat. Res.*, **16**, 1207-1216.

Chamberlain, G., Rosenthal, H. (1995). Aquaculture in the next century, opportunities for growth, challenges of sustainability. *World Aquacult.* **26**, 21-25.

Chen, S., Stechey, D., Malone, R. F. (1994). Suspended solids control in recirculating aquaculture systems. In: Timmons, M. B. and Losordo, T. M. (Eds.) Aquaculture water reuse systems: engineering design and management. Amsterdam, Netherlands. Elsevier Science: pp. 75-91.

Chen, S., Coffin, D. E., Malone, R. F. (1997). Sludge production and management for recirculating aquaculture systems. Journal of World Aquaculture Society, **28**(4): pp. 303-315.

Colt, J. E., Orwicz, K. (1991). Aeration in intensive culture. Pages 198-271 In: D.E. Brune and J.R. Tomasso (Eds.), *Aquaculture and Water Quality*. World Aquaculture Society, Louisiana State University, Baton Rouge, Louisiana.

Courtland, S. (2000).Fluidized bed biofiltration technology for oligotrophic water quality. Global Aquaculture Advocate. June 2000 Vol. 3 Issue 3.

Cox, C. R. (1964). Operational and control of water treatment processes. World Health Organization Geneva, 81.

Davis, J. A. (1980). Adsorption of natural organic matter from freshwater environments by aluminium oxide. In: Contaminants and Sediments, Vol. 2 (ed. R. A. BAKER) pp. 279-303. Ann Arbor Sci. Publ. Inc., Michigan.

Davis, J. A. (1982). Adsorption of natural dissolved organic matter at the oxide/water interface. *Geochim. Cosmochim. Acta*, **46**, 2381-2393.

de Beer, D., Stoodley, P., Roe, F., Lewandowski, Z. (1994). Effects of biofilm structures on oxygen distribution and mass transport. *Biotechnol. Bioengng*, **43**, 1131-1138.

Diab, S., Shilo, M. (1988). Effect of light on the activity and survival of Nitrosomonas sp. and Nitrobacter sp. isolated from fish ponds. *Israeli J. of Aquacult.*, **40**, 50-6.

Di Ruggero, J. (1989). Ecologie et physiologie des bactéries réduisant le manganése. Exemple de la nappe alluviale du Rhône, Avignon (Vaucluse). Thése Doct. Univ. Lyon I. In: Gounot, A. M. (1994). Microbial oxidation and reduction of manganese: Consequences in groundwater and applications. *Microbiology Reviews*, **14**, 339-350.

Doran, P. M., Bailey, J. E. (1986). Effects of immobilization on growth, fermentation, properties and macromolecular composition of *S. cerevisiae* attached to gelatin. *Biotechnol. Bioengng* **28**, 73-87.

Drennan, D. G., Rahman, M. M., Malone, R. F. (1993). Slow sand filtration technologies for the containment of non-indigenous species in recirculating aquaculture effluents. In Techniques for Modern Aquaculture – Proceedings of an Aquacultural Engineering Conference 21-23 June 1993. Spokane, Washington.

Drtil, M., Nemeth, P., Bodik, I. (1993). Kinetic constant of nitrification. *Water Res.*, **27**, 35-39.

Dubinina, G. A. (1979). Functional role of bivalent iron and manganese oxidation in *Leptothrix pseudoocharaceae*. *Microbiology USSR*, **47**, 631-636.

Duddles, G. A., Richardson, S. E., Barth, E. F. (1974). Plastic-medium trickling filters for biological nitrogen control. *J. Wat. Pollut. Control Fed.*, **46**, 937-946.

Eddy, F. B., Kunzlik, P. A, Bath, R. N. (1982). Uptake and loss of nitrite from the blood of rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L. in fresh water and in dilute seawater. *J. Fish Biol.*, **23**, 105-116.

Eddy, F. B., Williams, E. M. (1987). Nitrite and Freshwater Fish. *Chemistry and Ecology*, 1987, Vol. **3**, pp. 1-38.

Ehrilich, H. L. (1976). *Environ. Biogeochem.*, **2**, 633. In: Nealson, K. H., Tebo, B. M., Rosson, R. A. (1988). Occurrence and mechanisms of microbial oxidation of manganese. *Advance in Applied Microbiology*, **33**, 279-318.

Exley C., Phillips, M. J. (1988). Acid Rain: Implications for the Farming of Salmonids. In *Recent Advance in Aquaculture*, ed. J. F Muir & R. J. Roberts. CROOM HELM London & Sydney – TIMBER PRESS, Portland, Oregon, pp. 225-343.

FAO, 1997. Review of the state of world aquaculture. FAO Fisheries Department, FAO Fisheries Circular, 886, Rev. 1, FAO, Rome, 163 pp.

Fenton, D. R., Mills, P. J. (1980). On farm aerobic treatment of piggery wastes. The effect of residence time and storage on effluent quality. *Water Res.*, **14**, 805-08.

Figueroa, L. A., Silverstein, J. (1992). The effect of particulate organic matter on biofilm nitrification. *Water Environ. Res.*, **64**, 728-733.

Fitch, M.W., Pearson, N., Richards, G., Burken, J.G. (1998). Biological Fixed-Film Systems. *Water Environment Research*, **70**, 495.

Focht, D. D., Verstraete, W. (1977). Biochemical ecology of nitrification and denitrification. In *Advance in Microbial Ecology*, ed. M. Alexander. Plenum Press, New York, pp. 135-214.

Forster, J. R. M. (1974). Studies on nitrification in marine biological filters. Aquaculture 4, 387-397.

Franklin, B., Pilkington, D. (2001). The refurbishment of existing rapid gravity filters to upgrade performance; techniques used, constraints encountered and improvements achieved. In: International conference - Advances in rapid granular filtration in water treatment, 229-240.

Frijlink, M. J., Abee, T., Laanbroek, H. J., de Boer, W., Konings, W. N. (1992). The bioenergetics of ammonia and hydroxylamine oxidation in *Nitrosomonas europea* at acid and alkaline pH. *Arch. Microbiol.*, **157**, 194-199.

Froelick, P. N., Klinkhammer, G., Bender, M. L., Luedtke, N. A., Health, G. R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., Maynard, V. (1979). Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim. Cosmochim. Acta*, **43**, 1075-1090. In: Nealson, K. H., Myers, C. R. (1992). Microbial reduction of manganese and iron: new approaches to carbon cycling. *Applied and Environmental Microbiology*, **58** (2), 439-443.

Gabano, J. P., Etienne, P., Laurent, J. F. (1965). Electrochim. *Acta*, **10**, 947. In: Posselt H. S., Anderson F. J., Weber W. J. (1968). Cation sorption on colloidal hydrous manganese dioxide. *Environmental Science and Technology*, **2**, 12.

Gempesaw, C. M., Supitaningsih, J. R., Bacon, J. M., Hankins, J. A. (1993). Economic Analysis of an Intensive Aquaculture Recirculating System for Trout Production. Pages 263-277. In: J-K. Wang, Editor. Techniques for Moder Aquaculture. American Society of Agricultural Engineers, St. Joseph, Michigan.

Ghiorse, W. C. (1984). Biology of iron and manganese-de-positing bacteria. *Annu. Rev. Microbiol.*, **38**, 515-550. In: Gounot, A. M. (1994). Microbial oxidation and reduction of manganese: Consequences in groundwater and applications. *Microbiology Reviews*, **14**, 339-350.

Gibbs, M., Schiff, J. A. (1960). Chemosynthesis: The energy relations of chemoautotrophic organisms. In: Steward, F. C. (Ed.), Plant Physiology: A Treatise, Vol. IB: photosynthesis and chemosynthesis. Academic Press, New York, pp. 279-319.

Golz, W. J., Rusch, K. A., Malone, R. F. (1996). Developing backwash protocols for floating-bead filters: a model of solid-loading and biofilm-retention effects on nitrification. In: Successes and Failures in Commercial Recirculating Aquaculture: Proceeding of the Aquacultural Engineering Society, Virginia Polytechnic Institute/Northeast Regional Agricultural Engineering Service, Roanoke, Virginia, 196-205.

Golz, W. J., Rusch, K. A., Malone, R. F. (1999). Modeling the major limitations on nitrification in floating-bead filters. *Aquacultural Engineering*, **20**, 43-61.

Gounot, A. M. (1994). Microbial oxidation and reduction of manganese: Consequences in groundwater and applications. *Microbiology Reviews*, **14**, 339-350.

Grace, G. R., Piedrahita, R. H. (1993). Carbon dioxide control with a packed column aerator. Pages 496–505 In: J. K. Wang (Ed.), *Techniques for modern aquaculture*. American Society of Agricultural Engineers, Saint Joseph, Michigan

Grace, G. R., Piedrahita, R. H. (1994). Carbon dioxide control. Pages 209–234 In: M. B. Timmons and T. M. Losordo (Eds.), *Aquaculture water reuse systems: Engineering*

Grady, C. P. L., Lim, H. C. (1980). Biological wastewater treatment. New York, New York: Marcel Dekker. In: Timmons, M. B., Losordo, T. (1994). Operating and Managing Water Reuse Systems. Engineering Design and Management. Elsevier: Developments in Aquaculture and Fisheries Science, Vol. 27

Gramm-Osipov L. M., Volkova T. I., Chichkin R.V. (1991). Laboratory data on the behaviour on manganese dioxide in aqueous solution. *Geochemistry-International*, **29(7)**, 28-36.

Gujer, W., Boller, M. (1986). Design of nitrifying tertiary trickling filter based on theoretical concepts. *Water Research*, 20 (11), 1353-1362. In: Timmons, M. B., Losordo, T. (1994). Operating and Managing Water Reuse Systems. Engineering Design and Management. Elsevier: Developments in Aquaculture and Fisheries Science, Vol. 27.

Hagopian, D. S., Riley, J. G. (1998). A closer look at the bacteriology of nitrification. *Aquacultural Engineering*, **18**, 223-244.

Harremoes, P. (1978). Biofilm kinetics. In *Water Pollution Microbiology, Vol. 2*, ed. R. Mitchell. John Wiley, New York, pp. 71-109.

Hattori, R., Edeline, F. (1981). Growth rate and growth yield of *E. coli* adsorbed on an anion exchanger resin. *J. gen. appl. Microbial.* **27**, 287-298.

Haug, R.T., McCarty, P.L. (1972). Nitrification with submerged filters. Journal Water Pollution Control Federation 44(11): 2086-2102.

Heinsbroek, L. T. N., Kamstra, A. (1990). Design and performance of water recirculation systems for eel culture. *Aquacultural Engineering*, **9**, 187-207.

Holben, W. E., Noto, K., Sumino, T., Suwa, Y. (1998). Molecular analysis of bacteria communities in a three-component granular activated sludge system indicates community-level control by incompatible nitrification processes. *Appl. Env. Microbiol.*, 64 (7), 2528-2532.

Hollocher, T. C., Tate, M. E., Nicholas, D. J. D. (1981). Oxidation of ammonia by *Nitrosomonas europaea*. Definitive 18O-tracer evidence that hydroxylamine formation involves a monooxygenase. J. Biol. Chem. 256, 10834-10836.

Honer, G., Rosenthal, H., Kruner, G. (1985). Rearing of juvenile Sarotherodon galilaeus in laboratory aquaria. I. Fluctuation of water quality. ICES (Council Meeting) C.M. 1985/F:66, 1 – 17.

Hopper, A. B. (1989). Biochemistry of the nitrifying lithoautotrophic bacteria. In: Schlegel, H. G., Bowien, B. (Eds), Autotrophic Bacteria. Science Tech, Madison, WI, pp. 239-265.

Horn, H. (1994). Dynamics of a Nitrifying Bacteria Population in a Biofilm Controlled by an Oxygen Microelectrode. *Water Science Technology*, **29**,69.

Horowitz, A., Horowitz, S. (2000). Improving biofiltration in Recirculating Aquaculture Systems. *The Advocate*, 70-71.

Housecroft, C. E., Constable, E. C. (2002). Chemistry. 2nd Edition. Pearson Education UK.

Hovanec, T. A., DeLong, E. F. (1996). Comparative analysis of nitrifying bacteria associated with freshwater and marine acquaria. *Appl. Env. Microbiol.*, 62 (8), 2888-2896.

Hunter, K. A. (1980). Microelectrophoretic properties of natural surface-active organic matter in coastal seawater. *Limnol. Oceanogr.*, **25**, 807-822.

Kaiser, G. E., Wheaton, F. W. (1983). Nitrification filters for aquatic culture systems: state of the art. *Journal of the World Mariculture Society*, **14**, 302-324.

Kamstra, A., van der Heul, J. W., Nijhof, M. (1998). Performance and optimization of trickling filters on eel farms. *Aquacultural Engineering*, **17**, 175-192.

Kawashima, M., Tainaka, Y., Hori, T., Koyama, M., Takamatsu, T. (1986). Phosphate adsorption onto hydrous manganese (IV) oxide in the presence of divalent cations. Water Res., 20, 371-375.

Keen, G. A., Prosser, J. I. (1988). The surface growth and activity of *Nitrobacter*. *Microb. Ecol.*, **15**, 21-39.

Kirkup S. P., Marsh L. S., Libey G. S., Heath A. G., Coale C. W., Haugh C. G. (2000). Effects of Temperature and Feeding Rates Upon Growth and Feed Conversion Ratios in Tilapia. *International Conference on Recirculating Aquaculture*-Virginia Tech-Technical section 4-RAS Operation, 18.

Klein, J., Ziehr, H. (1990). Immobilization of microbial cells by adsorption. *J. Biotechnol.*, **16**, 1-16.

Kornegay, B. H., Andrews, J. F. (1969). Kinetics of fixed film biological reactor. *J. Wat. Pollut. Control Fed.*, **40**, 460-468.

Kozawa, A., J. (1959). Electrochem. Soc., 106, 552. In: Posselt H. S., Anderson F. J., Weber W. J. (1968). Cation sorption on colloidal hydrous manganese dioxide. *Environmental Science and Technology*, **2**, 12.

Kruner, G., Rosenthal, H. (1983). Efficiency of Nitrification in Trickling Filters using Different Substrates. *Aquacultural Engineering*, **2**, 49-67.

LaMotta, E. J. (1976). Kinetics of growth and substrate uptake in a biological film system. *Appl. Envir. Microbiol.*, **31**, 286-293.

Lawson, T. B. (1995). Fundamental of aquacultural engineering. 209-212, 215-238. USA: Chapman and Hall.

Lazarova, V., Manem, J. (1995). Biofilm characterization and activity analysis in water and wastewater treatment. Wat. Res. Vol. 29, No 10, pp. 2227-2245.

Liao, P. B., Mayo, R. D. (1974). Intensified fish culture combining water reconditioning with pollution abatement. *Aquaculture*, **3**, 61-85.

Loganathan, P., Burau, R. G. (1973). Sorption of heavy metals by hydrous manganese minerals. Geochim. Cosmochim. Acta, 37, 1277-1293. In: Balistrieri L. S., Murray J. W. (1982). The surface chemistry of δ-**MnO2** in major ion seawater. *Geochimica et Cosmochimica Acta*, **46**, 1041-1052.

Losordo, T. M., Westerman, P. W. (1991). An analysis of biological, economic, and engineering factors effecting the cost of fish production in recirculating aquaculture systems. *Design of high density recirculating aquaculture systems*. Baton Rouge: Louisiana Sea Grant Program, Louisiana State University, pp.1-9.

Lovley, D. R. (1991). Dissimilatory Fe (III) and Mn (IV) reduction. *Microbiological Reviews*, **55** (2), 259-287.

Manem, J. A., Rittmann, B. E. (1992). The effects of fluctuation in biodegradable organic matter on nitrification filters. *J. AWWA*, **84**, 147-151.

Metcalf, A., Eddy, A. (1991). Wastewater Engineering: Treatment, Disposal, Reuse, 3rd ed. Tchobanoglous, G., Burton, F. (Eds.), McGraw-Hill international, New York.

McKenzie, R. M. (1970). The reaction of cobalt with manganese dioxide minerals. Aust. J. Soil Res., 8, 97-106. In: Balistrieri L. S., Murray J. W. (1982). The surface chemistry of δ-MnO_2 in major ion seawater. *Geochimica et Cosmochimica Acta*, **46**, 1041-1052.

Mero, J. L. (1965). The Mineral Resources of the Sea. Elsevier, Amsterdam, 1965. In: Murray J.W. (1973). The surface chemistry of hydrous manganese dioxide. *Journal of Colloid and Interface Science*, **46**, 3.

Monita, D. (2002). Reliable, Low Cost On-Site Oxygen Generators. *International Conference on Recirculating Aquaculture*-Virginia Tech. Symposium 6 - Systems, pp.170.

Morgan J.J., Stumm W. (1964). Colloid-chemical properties of manganese dioxide. *Journal of Colloid Science*, **19**, 347-359.

Morgan J.J., Stumm W. (1965). *Colloid Sci.*, 19 (4), 347. In: Posselt H. S., Anderson F. J., Weber W. J. (1968). Cation sorption on colloidal hydrous manganese dioxide. *Environmental Science and Technology*, **2**, 12.

Mortensen, H. (2002). Microscreens, Efficiencies in Aquaculture Systems. *International Conference on Recirculating Aquaculture*-Virginia Tech. Symposium 6 - Systems, pp.163.

Mouchet, P. (1992). From conventional to biological removal of iron and manganese in France. *J. Am. Water Works Assoc.*, **84**, 158-162.

Muir, J. F. (1981). Management and Cost Implications in Recirculating Water Systems. Page 116-127. In: Bio-Engineering Symposium for Fish Culture (FCS Publ.1). American Fisheries Society, Bethesda, MD.

Muir, J. F. (1982). Recirculated water systems in aquaculture. In *Recent Advance in Aquaculture*, ed. J. F Muir & R. J. Roberts. Westview Press, Boulder, Colorado, pp. 358-446.

Muir, J. F. (1995). 'Many happy return? Water reuse systems in aquaculture'. Aquaculture toward the 21st Century. Kuala Lumpur. Infofish.

Muir, J. F. (1998). The European Experience with Production Intensification. *International Conference on Recirculating Aquaculture*-Virginia Tech-Coldwater Aquaculture Conference, pp.210.

Murray, D. J., Healy, T. W., Fuerstenau, D. W. (1968). Adsorption for Aqueous Solution. Advan. Chem. Ser., 79, In press. In: Posselt H. S., Anderson F. J., Weber W. J. (1968). Cation sorption on colloidal hydrous manganese dioxide. *Environmental Science and Technology*, **2**, 12.

Murray J.W. (1973). The surface chemistry of hydrous manganese dioxide. *Journal of Colloid and Interface Science*, **46**, 3.

Murray J.W. (1975). The interaction of metal ions at the manganese dioxide-solution interface. *Geochimica et Cosmochimica Acta*, **39**, 505-519.

Murray, J. M., Dillard, J. G. (1979). The oxidation of cobalt (II) adsorbed on manganese dioxide. *Geochim. Cosmochim. Acta*, **43**, 781-787. In: Balistrieri L. S., Murray J. W. (1982). The surface chemistry of δ-**MnO2** in major ion seawater. *Geochimica et Cosmochimica Acta*, **46**, 1041-1052.

Nealson, K. H., Tebo, B. M., Rosson, R. A. (1988). Occurrence and mechanisms of microbial oxidation of manganese. *Advance in Applied Microbiology*, **33**, 279-318.

Nealson, K. H., Rosson, R. A., Myers, C. R. (1989). Mechanisms of oxidation and reduction of manganese. In: Metal Ions and Bacteria (Beveridge, T. J. and Doyle, R. J., Eds.). pp. 383-411. Wiley-Interscience, New York, NY.

Nealson, K. H., Myers, C. R. (1992). Microbial reduction of manganese and iron: new approaches to carbon cycling. *Applied and Environmental Microbiology*, **58** (2), 439-443.

Nijhof, M. (1994a). Bacterial stratification and hydraulic loading effects in a plug-flow model for nitrifying trickling filters applied in recirculating fish culture systems. *Aquaculture*, 134 (1-2), 49-64.

Nijhof, M., Klapwijk, A. (1995). Diffusional transport mechanisms and biofilm nitrification characteristics influencing nitrite levels in nitrifying trickling filter effluents. *Wat. Res.*, Vol. 29, No. 10, pp. 2287-2292.

Nijhof, M. (1995). Bacteria stratification and hydraulic loading effects in a plug-flow model for nitrifying trickling filters applied in recirculating fish culture systems. *Aquaculture*, **134**, 49-64.

Olem, H., Unz, R. F. (1980). Rotating-disc biological treatment of acid mine drainage. *J. Wat. Pollut. Control Fed.*, **52**, 257-269.

Olson, R. J. (1981). Differential photo-inhibition of marine nitrifying bacteria: a possible mechanism for the formation of the primary nitrite maximum. *J. Mar. Res.*, **39**, 227-38.

O'Rourke, P. D. (1991). Current Status on Profits in Recirculating Systems. Page 65-99. In: Regional Workshop on Commercial Fish Culture Using Water Recirculating Systems. IL-IN-SG-E-91-8. Illinois/Indiana Sea Grant, Illinois State University, Normal, Illinois.

Otte, G., Rosenthal, H. (1979). Management of a close brackish water system for high-density fish culture by biological and chemical water treatment. *Aquaculture*, **18**, 169-81.

Painter, H. A. (1970). A review of literature on inorganic nitrogen metabolism in micro-organisms. *Wat. Res.*, **4**, 393-450.

Pano, A., Middlebrooks, E. J. (1983). Kinetics of carbon and ammonia nitrogen removal in RBC's. *Journal Water Pollution Control Federation*, **55**, 956-965.

Patrick, W. H., Jugsujinda, A. (1992). Sequential Reduction and Oxidation of Inorganic Nitrogen, Manganese, and Iron in Flooded Soil. *Soil Sci. Soc. Am. J.*, **56**, 1071-1073.

Perrone, S. J., Meade, T. L. (1977). Protective effect of chloride on nitrite toxicity to Coho salmon (*Onchorhyncus kisutch*). *Journal of the Fisheries Research Board of Canada*, **34** (4), 486-492.

Posselt H. S., Anderson F. J., Weber W. J. (1968). Cation sorption on colloidal hydrous manganese dioxide. *Environmental Science and Technology*, **2**, 12.

Poxton, M. G., Murray, K. R., Linfoot, B. T., Pooley, A. B. W. (1981). The design and performance of biological filters in an experimental mariculture facility. *Proc. World Symp. On Aquaculture in Heated Effluents and Recirculation Systems*, Stavanger, 28-30 May 1980.

Poxton, M.G., Allouse, S.B. (1987). Cyclical fluctuations in Ammonia and nitrite-nitrogen resulting from the feeding of turbot *Scophthalmus maximum* (L.), in recirculating systems. Aquacultural Engineering 6: 301 – 322.

Pyman, M. A. F., Posner, A. M. (1978). The surface area of amorphous mixed oxides and their relation to potentiometer titration. *J. Colloid. Interface Sci.* **66**, 85-94.

Rasmussen, K., Lewandowski, Z. (1998). Microelectrode Measurements of Local Mass Transport Rates in Heterogeneous Biofilms. *Biotechnology and Bioengineering*, **59**, 302.

Rittmann, B.E., McCarty, P.L. (1980). Model of Steady-State-Biofilm Kinetics.*Biotechnology and Bioengineering*, **22**, 2343.

Rogers, G. L., Klemetson, S. L. (1985). Ammonia removal in selected aquaculture water reuse biofilters. *Aquacultural Engineering*, **4**, 135-154.

Rosenthal, H. (1981a). Recirculation systems in western Europe. Proc. World Symp. On Aquacult. In Heated Effl. And Recirculation Systems. II (Berlin; Heenemann), pp. 305-315.

Rosenthal, H. (1993). The history of recycling technology: A lesson learned from the past experience? *Fish Farming Technology, Reinertsen, Dahle, Jorgensen, Tvinnereim (eds)*, 341-349.

Rosenthal, H. (1999). Biofiltration and the importance of biofilm structure. Recirc. Today. Vol. 1 – Issue 4 – July 1999.

Rosenthal, H., Andjus, R., Kruner, G. (1980). Daily variations of water quality parameters under intensive culture conditions in a recycling system. *EIFAC/80/Symp.: E/59.*

Rusin, P. A., Quintana, L., Sinclair, N. A., Arnold, R. G., Oden, K. L. (1991). Physiology and kinetics of manganese-reducing Bacillus polymyxa strain D1 isolated from manganiferous silver ore. *Geomicrobiol.*, 9, 13-25. In: Ghounot, A. M. (1994). Microbial oxidation and reduction of manganese: Consequences in groundwater and applications. *Microbiology Reviews*, 14, 339-350.

Russo, R. C., Thurston, R. V, Emerson, K. (1981). Acute Toxicity of Nitrite to Rainbow Trout (Salmo gairdneri): Effects of pH, Nitrite Species, and Anion Species. *Can. J. Fish. Aquat. Sci.*, Vol. 38, 1981.

Savenko, V. S. (1985). Geokhimiya, 3, 416. In: Gramm-Osipov L. M., Volkova T. I., Chichkin R.V. (1996). Laboratory data on the behavior on manganese dioxide in aqueous solution. *Geochemistry-International*, 29(7), 28-36.

Sawyer, C.N., McCarty, P.L., Parkin, G.F. (1994). *Chemistry for Environmental Engineering* (4th ed.). McGraw-Hill, New York, 658 pp.

Sharma, B., Ahlert, R. C. (1977). Nitrification and nitrogen removal. *Water Res.*, 11, 897-925.

Shilo, M., Rimon, A. (1982). Factors which affect the intensification of fish breeding in Israel. 2. Ammonia transformation in intensive fish ponds. Isr. J. Aquaculture Bamidgeh, 34 (3), 101-114.

Siegrist, H., Gujer, W. (1985). Mass transfer mechanisms in a heterotrophic biofilm. *Water Res.*, 19, 1369-1378.

Siegrist, H., Gujer, W. (1987). Demostration of mass transfer and pH effects in a nitrifying biofilm. *Water Res.*, 21 (12), 1481-1487.

Speece, R. E. (1973). Trout metabolism characteristics and the rational design of nitrification facilities for water re-use in hatchery. Trans. Am. Fish. Soc. 102, 323-332.

Spotte, S. (1979). Fish and invertebrate culture. Water management in closed systems. Second Ed., USA: Wiley – interscience. 0-471-02306-X.

Srna, R.F., Baggaley, A. (1975). Kinetic Response of Perturbed Marine Nitrification Systems. *Journal of the Water Pollution Control Federation*, 47, 472.

Stirling, A. P. (1985). Chemical and biological methods of water analysis for aquaculture. Institute of Aquaculture, University of Stirling. Edited by Hadrian P. Stirling.

Stone, A. T., Morgan, J. J. (1984). Reduction and Dissolution of Manganese (III) and Manganese (IV) Oxides by Organics: 2. Survey of the Reactivity of Organics. *Environmental Science & Technology*, **18**, 617-624.

Stumm, W., Morgan, J. J. (1981). Aquatic Chemistry. Wiley, New York.

Summerfelt, S. T., Hankins, J. A., Weber, A. L., Durant, M. D. (1997). Ozonation of a recirculating rainbow trout culture system. II. Effects on microscreen filtration and water quality. *Aquaculture*, **158**, 57-67.

Summerfelt, S. T., Wade, M. (1998). Fluidised-sand biofilters installed at two farms. Recirc Today 18-20.

Summerfelt, S. T., Davidson, J., Timmons, M. B. (2000). Hydrodynamics in the 'Cornell-type' dual-drain tank. In: Proceeding, Third International Conference on Recirculating Aquaculture. Pp 160-166.

Summerfelt, S.T., Vinci, B. J., Piedrahita, R. H. (2000a). Oxygenation and carbon dioxide control in water reuse systems. *Aquacultural Engineering*, 22: 87-108.

Summerfelt, S. T., Davidson, J., Waldrop, T., Vinci, B. J. (2002). Evaluation of Full-Scale Carbon Dioxide Stripping Columns in a Coldwater Recirculating System. *International Conference on Recirculating Aquaculture*-Virginia Tech-Water Quality Contributed Session, pp.439.

Swain, H. R., Lee, C, Rozelle, R. B. (1975). *Anal. Chem.*, **47**, 1135. In: Gramm-Osipov L. M., Volkova T. I., Chichkin R.V. (1996). Laboratory data on the behavior on manganese dioxide in aqueous solution. *Geochemistry-International*, **29(7)**, 28-36.

Tchobanoglous, G., Burton, F. (1991). *Wastewater Engineering: Treatment, Disposal, and Reuse* (3rd Edition.). McGraw-Hill, New York, 1334 pp.

Tetzlaff, B.L. (1991). "Suspended Solids Filtration in Water Recirculation Systems." *Second Annual Workshop on Commercial Aquaculture Using Water Recirculating Systems*, Nov.15-16.

Tetzlaff, B.L. and Heidinger, R. C. (1990). Basic Principles of Biofiltration and System Design. Illinois Aquaculture Resource/Research Center. Southern Illinois University, Carbondale, USA.

Timmons, M.B. 1994. "Use of foam fractionators in Aquaculture." pp 247-279. IN: *Aquaculture water reuse systems: engineering design and management*. M. B. Timmons and T. H. Losordo, Editors. Developments in Aquaculture and Fisheries Science, Vol. 27. Elsevier. New York.

Timmons, M. B., Losordo, T. (1994). Operating and Managing Water Reuse Systems. Engineering Design and Management. Elsevier: Developments in Aquaculture and Fisheries Science, Vol. 27.

Timmons, M. B., Aho, P. W. (1998). Comparison of aquaculture and broiler production systems. In: Proceeding, Second International Conference on Recirculating Aquaculture. NRAES Publication VA-1: 190-200.

Timmons, M. B., Summerfelt, S. T., Vinci, B. J. (1998). Review of circular tank technology and management. Aquacult. Eng. **18**, 51-69.

Timmons, M. B., Summerfelt, S. T. (1998). Application of fluidized-sand biofilters to Aquaculture. *International Conference on Recirculating Aquaculture*-Virginia Tech - AES TECH Section 2 – Biofiltration, pp.342-354.

Tipping, E. (1981a). The adsorption of aquatic humic substances by iron oxides. *Geochim. Cosmochim. Acta*, **45**, 191-199.

Tipping, E. (1981b). Adsorption of goethite (α-FeOOH) of humic substances from three different lakes. *Geochim. Cosmochim. Acta*, **33**, 81-89.

Tipping, E., Cooke, D. (1982). The effects of adsorbed humic substances on the surface charge of goethite (α-FeOOH) in freshwater. *Geochim. Cosmochim. Acta*, **46**, 75-80.

Tipping, E., Heaton, M. J. (1983). The adsorption of aquatic humic substances by two oxides of manganese. *Geochimica and Cosmochimica Acta*, **47**, 1393-1397.

Tsukuda, S. M., Hankins, C. P., Marshall, S. T., Summerfelt, G. L., Bullock, T. K. (1997). Effects of sand size on fluidized-bed biofilter performance in cold water systems. Pages 368-380 In: Timmons, M. B., Losordo, T. (Eds.) Recent Advances in Aquacultural Engineering (Proceedings). Northeast Regional Agricultural Engineering Service, Ithaca, NY.

Twaroska, J. G., Westerman, P. W., Losordo, T. M. (1997). Water treatment and waste characterization evaluation of an intensive recirculating fish production system. *Aquacultural Engineering*, **16**, 133-148.

Van Gorder, S. (2002). Design of a 65 Ton Recirculating system for high-valued species. *International Conference on Recirculating Aquaculture*-Virginia Tech-AES Section – Commercial Recirculating Systems, pp.300.

van Rjjn, J., Rivera, G. (1990). Aerobic and anaerobic biofiltration in an aquaculture unit-nitrite accumulation as a result of nitrification and denitrification. *Aquacultural Engineering*, **9**, 217-234.

Verhagen, F. J. M., Laanbroek, H. L. (1991). Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. *Appl. Env. Microbiol.*, 57 (11), 3255-3263.

Xambeau, I. (1990). Oxydation biologique du manganése par le bactéries, libres ou fixées, des eaux souterraines. These Doct. Univ. Lyon I. In: Gounot, A. M. (1994). Microbial oxidation and reduction of manganese: Consequences in groundwater and applications. *Microbiology Reviews*, **14**, 339-350.

Wade, E. M., Summerfelt, S. T., Hankins, J. A. (1996). Economies of scale in recycle systems. *International Conference on Recirculating Aquaculture*-Virginia Tech-AES Technical section 2: Open paper, pp.13.

Water Pollution Control Federation. (1983). Nutrient control, manual of practice. Publication Number FD-7. Washington, D.C. In: Timmons, M. B., Losordo, T. (1994). Operating and Managing Water Reuse Systems. Engineering Design and Management. Elsevier: Developments in Aquaculture and Fisheries Science, Vol. 27.

Watson, S. W. (1971). Taxonomic considerations of the family Nitrobacteraceae Buchanan. Int. J. Syst. Bacteriol. 21 (3), pp. 254-270.

Weeks, C.T., Westers, H. (2002). Manageability concerns in engineering and design of recirculating aquaculture systems. *International Conference on Recirculating Aquaculture*-Virginia Tech - Systems Contributed Session, pp.382.

Westerman, P. W., Losordo, T. M., Wildhaber, M. L. (1993). Evaluation of various biofilters in an intensive recirculating fish production facility. In Techniques for Modern Aquaculture – Proceedings of an Aquacultural Engineering Conference 21-23 June 1993. Spokane, Washington.

Wheaton, F. W. (2002). Recirculating Aquaculture Systems: An Overview of Waste Management. *International Conference on Recirculating Aquaculture*-Virginia Tech – Symposium 2 – Waste Management, pp.57.

Wilton, S. J. (2002). Design of a 50 T Salmon Smolt Operation. *International Conference on Recirculating Aquaculture*-Virginia Tech - AES Section – Commercial Recirculating Systems, pp.280.

Wortman, B. (1990). Effect of temperature on biodrum nitrification. Unpublished M. S. Thesis. University of Maryland. College Park, Maryland. In: Timmons, M. B., Losordo, T. (1994). Operating and Managing Water Reuse Systems. Engineering Design and Management. Elsevier: Developments in Aquaculture and Fisheries Science, Vol. 27.

Yao W., Millero F. J. (1996). Adsorption of phosphate on manganese dioxide in seawater. *Environmental Science and Technology*, **30**, No.2.

Yates, D. E. (1975). The structure of the oxides/aqueous electrolyte interface. Ph.D. Thesis, University of Melbourne, Australia. In: Balistrieri L. S., Murray J. W. (1982). The surface chemistry of δ-**MnO2** in major ion seawater. *Geochimica et Cosmochimica Acta*, **46**, 1041-1052.

Zhang, T. C., Fu, Y. C., Bishop, P. L. (1995). Competition for substrate and space in biofilms. *Water Environ. Res.*, **67**, 992-1003.

Appendix A
Brunauer-Emmett-Teller (BET) measurements

Water desorption measurements:

As the sample presented to the Micrometrics Germini II surface analyser must be dry it is treated as follows:

1) A clean, dry sample tube is weighed and between 0.5g and 1.0g of sample added.

2) The tube and contents placed in a heating block at a temperature greater than 100 °C, and heated for a least 2 hours with flowing nitrogen. The tube and contents are removed from the heating block and placed in a stand room temperature, with N_2 still flowing, and allowed to cool. They are then capped, re-weighed and the weight of dry sample calculated. The sample can now be presented to the instrument.

In the case of these samples an equal volume of sample was to be used rather than equal weights. The density of the sample was known therefore the volumes could be calculated.

Sample	Density (g/ml)	Wt as received (g)	Equivalent Volume (ml)	Wt after drying (g)	Equivalent volume (ml)	Wt after SA determination (g)
SG	1.003	0.800	0.798	0.790	0.788	0.791
SS	1.695	1.353	0.798	1.342	0.792	1.344
VR	0.660	0.527	0.798	0.494	0.748	0.495
MnO_2	1.956	1.561	0.798	1.524	0.779	1.524
MnO_2 (0.850-0.355 mm)	1.982	1.582	0.798	1.535	0.774	1.536
GAC	0.522	0.416	0.798	0.384	0.736	0.384

Isotherm and BET plot is given below as example for the MnO_2 media:

Appendix B
Material and methods used for water analysis

Ammonia measurements:

Materials: a spectrophotometer (Kontron Instruments: Uvikon 810), flasks with cork, phenol-nitroprusside buffer reagent, alkaline hypochlorite reagent and ammonia stock solution. The compounds were supplied by Aldrich Chemical Company (Aldrich).

Phenol-nitroprusside buffer reagent: dissolved 30 g sodium phosphate, Na_3PO_4. $12H_2O$, 30 g sodium citrate, $Na_3C_6H_5O_7$. $2H_2O$ and 3 g ethylenediamine tetra-acetic acid, disodium salt (Na_2 EDTA) in double distilled H2O and make up to 1 litre. Dissolved 60 g phenol, C_6H_5OH and 0.2 g sodium nitroprusside, $Na_2[Fe(CN)_5NO]$. $2H_2O$ in this solution.

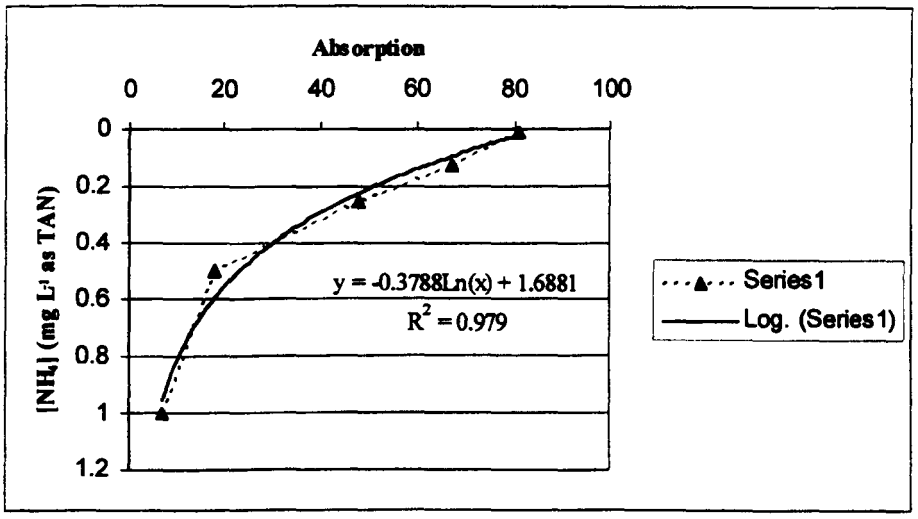
Alkaline hypochlorite reagent: add 30 ml commercial bleach solution (sodium hypochlorite, NaClO, containing 10 – 14% available chlorine to 400 ml 1 M NaOH and dilute with double distilled H_2O to 1 litre.

Procedure: A few standards from the stock solution and two blanks have to be prepared to obtain the standard curve. In 25 ml of filtered sample, double distilled water (blank) or standard solution, 10 ml of phenol-nitroprusside reagent are added and mix. Then, 15 ml of the hypochlorite reagent are added and mix. Each flask is closed with a cork and let to stand in the dark for 1 hour at room temperature. Finally, the adsorbance against the blank at 635 nm is measured with the spectrophotometer.

From the equation obtained with the standard curve, the ammonia concentration (as NH_4-N) in the sample is found.

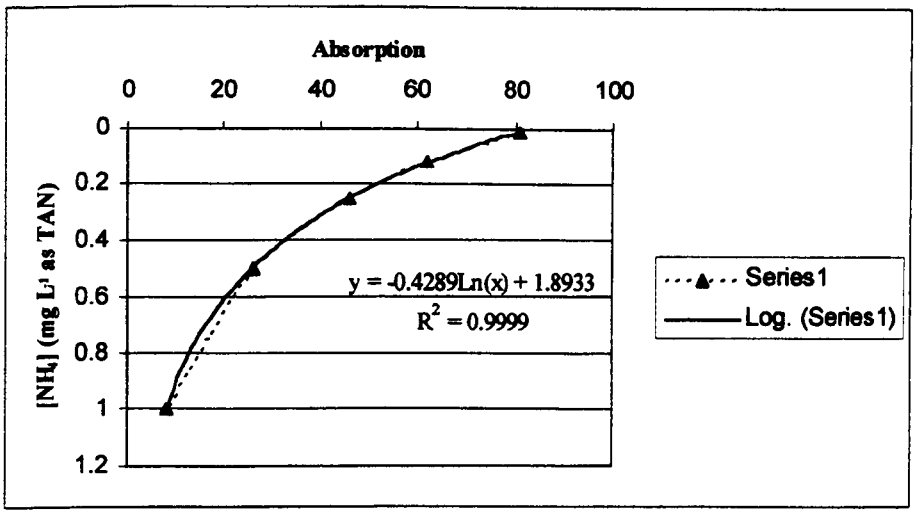
Standard calibration curve:

Abs.	81	67	48	18	7
Conc.	0.01	0.125	0.25	0.5	1



Palintest standard calibration curve:

Abs.	81	62	46	26	8
Conc.	0.01	0.125	0.25	0.5	1



Nitrite measurements:

Materials: a spectrophotometer (Kontron Instruments: Uvikon 810), conical flasks, sulphinamide solution, N-1 naphtylethylenediamine dihydrochloride (NED) solution, and nitrite stock solution. The compounds were supplied by Aldrich Chemical Company (Aldrich).

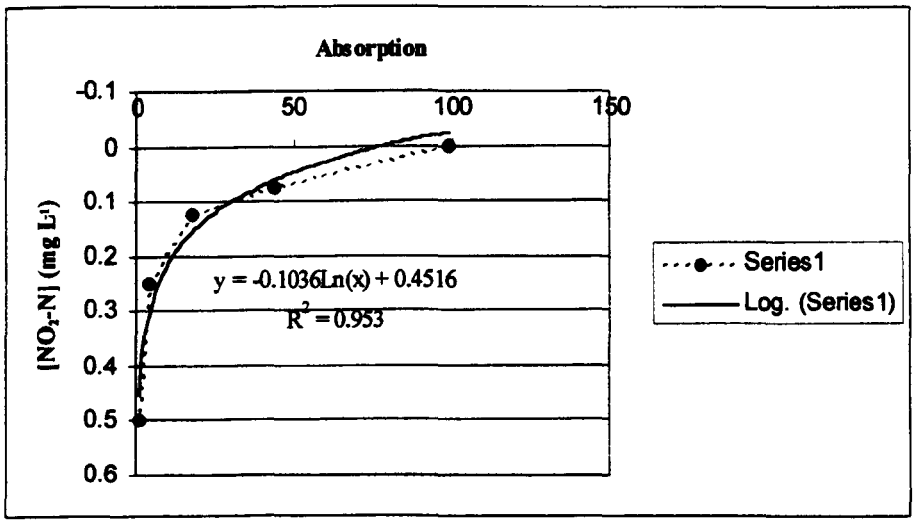
Sulphinamide solution: dissolve 5 g sulphinamide, NH2. C_6H4. SO_2. NH_2 in a mixture of 50 ml concentrated (12 M) HCl and 300 ml distilled H_2O. Dilute to 500 ml with double distilled H_2O.

N-1 naphtylethylenediamine dihydrochloride (NED) solution: dissolve 0.50 g of NED in 500 ml double distilled H_2O.

Procedure: A few standards from the stock solution and two blanks have to be prepared to obtain the standard curve. In 25 ml of filtered sample, double distilled water (blank) or standard solution, 0.5 ml of sulphinamide solution is added and mixed. After 2 – 8 minutes, 0.5 ml of NED solution is added and mixed. The prepared samples are left for 10 minutes to allow the colour to develop. Then, the adsorbance against the blank at 540 nm is measured with the spectrophotometer. From the equation obtained with the standard curve, the nitrite concentration (as NO_2-N) in the sample is found.

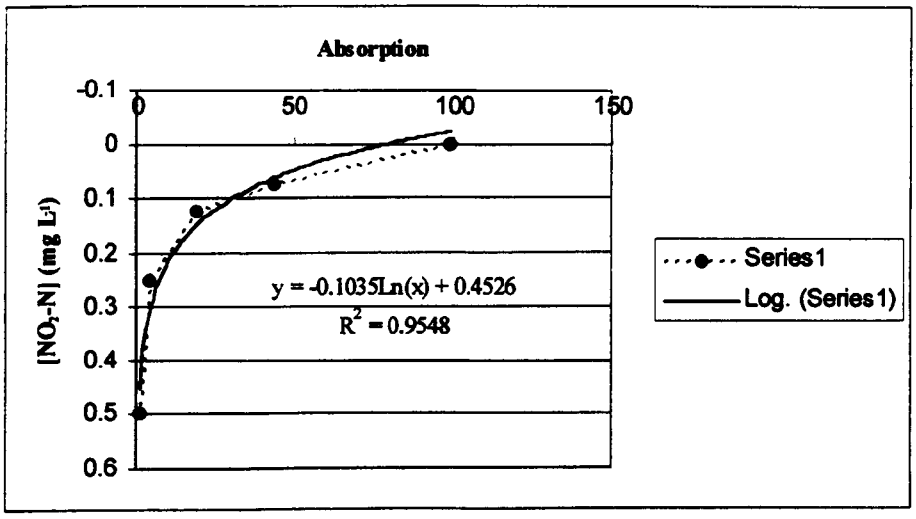
Standard calibration curve:

Abs.	99	43	18	4	1
Conc.	0	0.075	0.125	0.25	0.5



Palintest standard calibration curve:

Abs.	99	43	19	4	1
Conc.	0	0.075	0.125	0.25	0.5



Nitrate measurements:

Materials: a cadmium/copper column constructed from glass, 50 ml measuring cylinders, 2 M hydrochloric acid, copper sulphate solution, buffer solution, cadmium metal filings, and nitrite stock solution. The compounds were supplied by Aldrich Chemical Company (Aldrich):

2 M hydrochloric acid: add 16.7 ml concentrated (12 M) HCl to 50 ml double distilled water and make up to 100 ml.

Copper sulphate solution: dissolve 20 g copper sulphate, $CuSO_4$. $5H_2O$ in 1 litre double distilled H_2O.

Buffer solution: dissolve 100 g ammonium chloride, NH_4Cl, 20 g sodium borate, $Na_2B_4O_7$ and 1 g ethylenediamine tetra-acetic acid, disodium salt (Na_2 EDTA) in distilled water and make up to 1 litre.

Cadmium metal filings: use filings that pass through a 2.0 mm mesh sieve but are retained on a 0.5 mm mesh size.

Procedure: Prepare the column by washing 20 g cadmium filings with 100 ml 2 M HCl and then rinse with distilled water. Add 40 ml copper sulphate solution and swirl the content until the blue colour disappeared. Plug the base of the column with glass wool and add treated cadmium. Flush the column twice with 50 ml distilled water plus 5 ml buffer solution.

A few standards from the stock solution and two blanks have to be prepared to obtain the standard curve. In 50 ml of filtered sample, double distilled water (blank) or standard solution, 5 ml of buffer solution are added and mix. Place 20 ml of this solution on the column and allow it to run through to waste, adjusting the flow to deliver 25 ml in 240 ± 10 sec. Add the rest of the sample to the column and then collect 25 ml sample, which is then analysed for nitrite as described n the previous section.

Suspended solids measurements:

Materials: Filter funnel (Volume = 1 L), vacuum source (Venturi suction pump), 47-mm Whatman GF/C glass-fibre papers, microbalance (Mettler: BB 2400), aluminium foil, dryer.

Procedure: Water samples (Volume = 1 L) are filtered under vacuum through pre-weighed filter papers. Then transfer the filter papers to aluminium foil and dry it for 24 hr at 75 °C. The increase in weight after drying gives the quantity of inorganic and organic solids in the volume of water filtered.

B.O.D measurements:

Materials: Oxygen meter YSI model 58 and BOD probe YSI

Procedure: Three water samples from each test are collected in 300 ml glass bottles. The oxygen content is determined immediately in one of these samples, while the other two samples are stopped and placed in an incubator in the dark for 5 days. At the end of this time, the dissolve oxygen is determined and the difference between the initial oxygen value and the final one gives the B.O.D value. The oxygen uptake of a sample is proportional to the quantity of organic material present.

Appendix C
Batch trials results

The results of the metabolite adsorption and reaction batch trials are presented. The values are the means of two replicates.

Test batch/Standard solution.						
	DO	**Temp.**				**Mn^{2+}**
Day 1	**(mg/L)**	**C**				**(ug/L)**
Time A	7.7	24.7				4.5
Time C	8	25.4				205.1
Time F	8.1	24.9				140.6
Control batch/10 ppm NH_4-N.						
	DO	**Temp.**	**NH_4-N**		**NO_3-N**	
	(mg/L)	**C**	**(mg/L)**		**(mg/L)**	
Day 1						
Time A	7.78	25.1	10.07		0	
Time C	8.39	24.5	0.18		0.02	
Time F	8.4	24.6	3.18		0.02	
Day2						
Time G	8.36	24.9	0.02		0	
Time I	8.42	24.9	4.52		0.02	
Time L	8.44	24.9	2.23		0.04	
Test batch/10 ppm NH_4-N.						
	DO	**Temp.**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Mn^{2+}**
	(mg/L)	**C**	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(ug/L)**
Day 1						
Time A	7.92	24.1	9.76	0	0	2.8
Time C	8.39	24.1	0.05	0	0.1	221.7
Time F	8.36	23.8	2.42	0	0.01	273.9
Day2						
Time G	8.01	23.5	0.04	0	0	3.1
Time I	8.1	23.1	2.35	0	0.41	276.7
Time L	8.06	23.3	1.71	0	0.07	165.5
Test batch/5ppm NH_4-N.						
	DO	**Temp.**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Mn^{2+}**
	(mg/L)	**C**	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(ug/L)**
Day 1						
Time A	8.23	24.3	4.77	0	0	0
Time C	8.17	24.1	0.18	0	0.12	218.2
Time F	8.08	24.2	0.44	0	0.06	272.7
Day2						
Time G	8.16	25	0.01	0	0	2.66
Time I	7.91	24.5	0.56	0	0.43	424.4
Time L	7.96	24.4	0.69	0	0.16	200
Pure Mn batch/5ppm NH_4-N.						
	DO	**Temp.**	**NH_4-N**	**NO_2-N**	**NO_3-N**	
	(mg/L)	**C**	**(mg/L)**	**(mg/L)**	**(mg/L)**	
Day 1						
Time A	8.21	24.3	4.01	0	0	
Time C	8.16	24.1	0.53	0	0.1	
Time F	8.05	24.2	1.56	0	0.05	

Control batch/10 ppm NO_2-N.						
	DO (mg/L)	Temp. C		NO_2-N (mg/L)		
Day 1						
Time A	7.12	24.9		9.11		
Time C	7.92	25.2		7.72		
Time F	8.01	24.6		9.11		
Day2						
Time G	7.15	24.8		0		
Time I	8.03	24.3		1.43		
Time L	8.08	24.4		0.02		
Test batch/10 ppm NO_2-N.						
	DO (mg/L)	Temp. C	NH_4-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	Mn^{2+} (ug/L)
Day 1						
Time A	8.46	22.8	0.03	9.25	0	1.9
Time C	8.63	22.3		0	0.08	345.4
Time F	8.64	22.3		0.41	6.75	422.9
Day2						
Time G	7.92	23.1		0	0	1.8
Time I	7.84	22.5		0	9.1	638.8
Time L	7.9	23.3		0	1.1	359
Test batch/5 ppm NO_2-N.						
	DO (mg/L)	Temp. C	NH_4-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	Mn^{2+} (ug/L)
Day 1						
Time A	8.38	23	0.03	4.71	0	1.2
Time C	8.47	22.3		0	0.08	161.1
Time F	8.45	22.2		0.62	4.03	210.5
Day2						
Time G	7.61	20.6		0	0	1.7
Time I	7.53	20		0	4.95	374.9
Time L	7.54	19.8		0	0.82	224.4
Control batch / 10 ppm NO_3-N.						
	DO (mg/L)	Temp. C	NH_4-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	
Day 1						
Time A	8.58	24.3	0.02	0	9	
Time C	8.47	24.1			0.4	
Time F	8.35	24.5			1.5	
Day2						
Time G	7.51	24.2			0.01	
Time I	7.43	24.5			8	
Time L	7.44	24.3			6.3	
Test batch / 10 ppm NO_3-N.						
	DO (mg/L)	Temp. C	NH_4-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	
Day 1						
Time A	8.37	23	0.04	0	9	
Time C	8.44	22.3			0.17	
Time F	8.41	22.5			2	
Day2						
Time G	7.66	20.6			0.01	
Time I	7.54	20			7.5	
Time L	7.51	20.5			7.1	

Appendix D
Pressurised filter characteristics

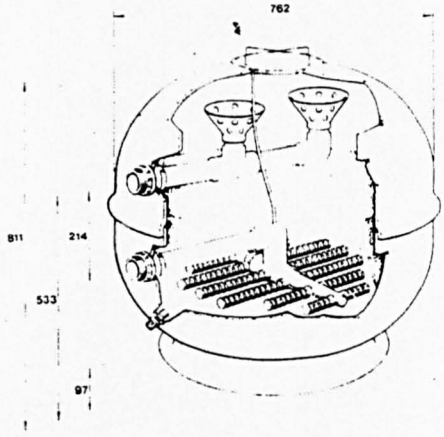
LACRON

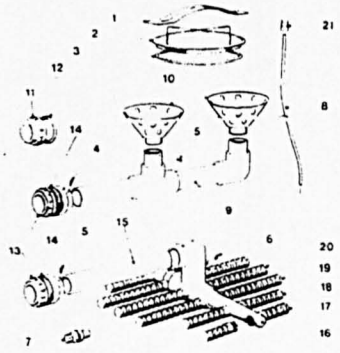
Hi-Rate Sand Filter

TYPE STANDARD: 762-(30"

DIMENSIONS AND SPARE PARTS

(All dimensions in mm)

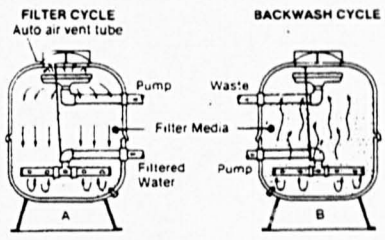




STOCK NO	DRAWING NO	DESCRIPTION		NO OFF
6012	LA30/1	Lid Tool	(1 per bag)	1
6000	LA30/2	Lid	(1 per bag)	1
6011	LA30/3	'O' Ring	(1 per bag)	1
1672	LA30/4	1" Self Tap Screw		1
1671	LA30/5	3/4" Self Tap Screw		2
1670	LA30/6	½" Self Tap Screw		1
6001	LA30/7	Drain Plug	(1 per bag)	1
17103	LA30/8	Air Bleed		1
17158	LA30/9	Main Header Less Rose		1
1317	LA30/10	Rose Distributor		2
6022	LA30/11	Adaptor Union	(2 per bag)	2
6005	LA30/12	Adaptor Gasket	(2 per bag)	2
6014	LA30/13	Bulkhead Fitting	(1 per bag)	2
6016	LA30/14	Bulkhead Gasket	(2 per bag)	2
17159	LA30/15	Main Collector Pipe & U/Drain Assembly less Candles		1
6007	LA30/16	Self Lock Screw Candle (120mm)(bagged)		4
6008	LA30/17	Self Lock Screw Candle (180mm)(bagged)		4
6009	LA30/18	Self Lock Screw Candle (240mm)(bagged)		6
1656	LA30/19	Air Bleed Cap		1

Lacron filters are suitable for removing suspended dirt particles from water a high speed efficiently and economically, in both fresh and salt wate conditions. Lacronfilters are constructed from totally corrosion resistan materials to ensure trouble free running and prolonged life. Each filter i rigorously pressure tested to the highest standards and certified as correc prior to leaving the factory.
The recommended media for Lacron filters is 16-30 grade silica sand to achiev excellent results.

FILTRATION PRINCIPAL



IMPORTANT

Prior to running check that all valves are in the correct positions. NEVEF attempt to alter valves without first stopping the pump – or in anywa subjecting the system to a closed head situation.

FILTER CYCLE

Contaminated water is pumped in through the top inlet port and distributed vi the head pipework system. Thus creating a uniform flow through the media be Dirt particles suspended in the water will penetrate and become embedded i the media, allowing the cleaned water to pass through fine slots in the collecti assembly. The water is then returned to the pool via pipework connected to th bottom outlet port. The effectiveness and efficiency of the filter is impaired b excessive build up of debris, clogging the media, which will result in pressur build up and poor circulation.
In this respect, we recommend that a suitably calibrated pressure gauge i installed on the pressurised pipework between the pump and the filter.
Increases in the normal running pressure of approximately 25-30 per cent wi indicate that BACKWASHING or cleaning of the media is required.

BACKWASH CYCLE

Backwashing or media cleaning is achieved by reversal of the water flo through the filter to waste and is activated by re-positioning the valves. It most important that water used on the backwash run is free from algae ar debris. Waste pipework should be kept as short as possible with a bore size th same as or greater than the filter inlet-outlet ports. Any restrictions or pipewo bends will reduce the efficiency of the backwash procedure.
The effect of backwashing can be clearly observed through the filter lid ar when first commenced the purged water will become extremely cloud Gradually this will change and after 2-3 minutes the water will clear completel leaving the media free of debris, at which point the backwash procedure can t stopped and the system re-set for the normal run or rinse cycle.

RINSE CYCLE

This may not be incorporated on all systems, but is designed to level the med bed and expel any foreign particles from inside the collector assembly. Th flow through the filter is in the same direction as for the normal filter cycle wi water used being exhausted to waste, via the valves and pipework.
The rinse cycle only requires 10-20 seconds running time to produce the corre results.

LACRON FILTER—762-30" SPECIFICATIONS

	IMPERIAL	METRIC
Overall Height	32"	811 mm
Overall Width	30"	762 mm
Unladen Weight	94 lbs	43 kilos
Face Pipework	2" B.S.P.	60 mmØ
Filtration Area	4.9ft²	0.46 m²
Design Working Pressure	15 P.S.I.	1 BAR
Maximum Working Pressure	22 P.S.I.	1.5 BAR
Test Pressure (Surge & Static)	60 P.S.I.	4 BAR
Media Content (16/30 Grade Silica Sand)	4 CWT	200 Kg
MAXIMUM FLOW RATE	**4900G.P.H.**	**22.8m³P.H**