The Use of a Laboratory-Scale Peat Biofilter to Study the Removal of Gaseous Ammonia

A thesis submitted to Dublin City University for the fulfilment of the requirements for the award of Masters of Science.

By

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I hereby certify that this material, which I now submit for the assessment on the programme of study leading to the award of M.Sc. is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: _____

ID No: _____

Date: _____

For my Family

Thank you so much for everything – words can not describe.

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ABSTRACT

The use of a laboratory scale peat biofilter to study the removal of gaseous ammonia.

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A laboratory scale biofilter was designed to study the removal of gaseous ammonia. The filter was constructed from perspex - 0.6 m in height and 0.2 m inner diameter. Peat granules were used as the packing material. The biofilter was operated at ambient temperature. Ammonia was supplied to the system in a discontinuous mode. The moisture level in the system was maintained at 40 – 60% by humidification of the inlet gas stream and by sprinkling the peat bed with water. The pH of the system fluctuated between pH 6.10 and pH 8.98. There were three stages of biofilter operation, during which ammonia was supplied discontinuously at concentrations of 14 mg m^{-3}, 565 mg m^{-3} and 2260 mg m^{-3} respectively. Ammonia was removed from the air with at least 90% efficiency even when high loads were supplied. However, up to 40 % of the ammonia supplied was removed by adsorbance on to the peat. There was also evidence of nitrification.

Although bacteria and fungi were detected on the native peat there were no nitrifiers and therefore the peat was inoculated with nitrifying activated sludge. Throughout biofilter operation peat samples were taken from the top of the filter and from sample ports at 0.2 m and 0.4 m of the filters' height for microbial analysis. The total bacterial counts increased from 10^5 cfu g^{-1} peat to 10^7 cfu g^{-1} peat. However, the numbers of both fungi and nitrifying bacteria decreased overall.

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1.0 INTRODUCTION

According to van Groenestijn and Hesselink (1993) most industrial and agricultural sectors, transport functions and energy production systems generate gaseous emissions, which are often of a polluting nature. Emissions from such operations include volatile organic compounds and oxidisable inorganic compounds, many of which give rise to odour, causing considerable nuisance even if they do not directly endanger health or the environment (Ottengraf, 1987). Other local effects of gaseous emissions include health problems, crop damage and smog formation. Depletion of the ozone layer and formation of acid rain are among the long-term effects associated with gaseous pollution. Over the last few decades there has been increased interest in gaseous emissions due to the deleterious effects on the environment and complaints of odour nuisance. The increase in interest has led to the implementation of more stringent national and international regulations for the control of gaseous emissions which in turn has led to an increase in research into abatement technologies (Leson and Winer, 1991).

1.1 Ammonia as a pollutant

Together with sulphur, ammonia is one of the principle acidifying pollutants in Europe (Cowell and ApSimon, 1998). It is also associated with odour nuisance, especially from intensive farming industries (Chen *et al.,* 1994). Gaseous ammonia is not persistent in the atmosphere, it only remains in the atmosphere for 5 – 10 days, after which it is deposited or chemically altered and is therefore an immediate source of pollution (http://h2osparc.wq.ncsu.edu/info/nh3.3html 05-01-2001). However, ammonia alone is not responsible for all nitrogenous pollution, nitric oxide (NO) and nitrogen dioxide (NO_2) as dry depositions and nitrate (NO_3^-) and ammonium (NH_4^+) as wet deposition also contribute to excessive nitrogen levels in any given environment (Stulen et al., 1998).

1.1.1 Ammonia characteristics

Ammonia is a colourless gas with a very pungent odour. It has a molecular weight of 17.03 mol g^{-1} and specific gravity of 0.597 at 70 F. It is the oldest commercial refrigerant and is still in use today, however it is most extensively used as soil fertiliser. It is irritating to the mucous membranes and the eyes. In its pure form it

can cause severe burns (http://www.c-f-c.com/specgas_products/ammonia.htm 05-01-2001).

According to the Merck Index its lower limit of human perception is 0.04 g m^{-3} or 53 mg L^{-1} (Windholz *et al.,* 1976). Gaseous ammonia has a TLV-TWA of 25 ppm, where TLV-TWA is the threshold limit value based on the weighed time average (http://www.c-f-c.com/specgas_products/ammonia.htm). The time weighted average is the average time over a given work period (e.g. an 8-hr work day) of a person's exposure to a chemical or an agent. The average is determined by sampling for the contaminant throughout the time period (http://home.att.net/~cobusters1/tvl.htm 05-01-2001). Safe levels for human health are given as TLV - TWA values.

1.1.2 Sources of gaseous ammonia emissions

There are natural sources of ammonia emissions, including human and animal excreta and bacterial generation. However anthropogenic emissions of nitrogen containing pollutants far exceeds natural emissions in Europe and North America (Stulen *et al.,* 1998). Anthropogenic sources include intensive livestock farming (animal wastes and fertilisers) and industrial processes e.g. coal conversion to coke, metallurgic operations, ceramics production, strip mining, chemical synthesis (nitric acid and plastics), waste gas treatment, sewage treatment plants, ammonium nitrate explosives production, production of refrigeration equipment, production of household cleaners, oil refineries and food processing (Sutton *et al.,* 2000). Ammonia emissions from livestock industries have been widely studied and ammonia is known to be present in the atmosphere surrounding pig units. The actual reported concentrations of ammonia identified in livestock buildings vary from study to study (Table 1).

Table 1 Ammonia concentrations identified in livestock buildings

Ammonia Concentration	Author
5 – 18 ppm (3.8 – 13.7 mg m^{-3})	Phillips *et al.*, (1998).
5 – 18 ppm (3.8 – 13.7 mg m^{-3})	Groot Koerkamp *et al.* (1998)
5 – 18 ppm (3.8 – 13.7 mg m^{-3})	Wathes *et al.*, (1998).
6 – 17 ppm (4.6 – 12.9 mg m^{-3})	van Langenhove *et al.*, (1988).
10.48 μg m^{-3}	M^{c}Cullock *et al.*, (1998)
*1 – 24 mg m^{-3}	van Geelen and van der Hoek, (1982).
0.1 – 18 mg m^{-3}	Klarenbeek, (1982).
0.01 – 1.9 mg m^{-3}	Kowelewsky *et al.*, (1980).
2.8 – 15.3 mg m^{-3}	Hilliger and Hartung, (1978).
18 mg m^{-3}	Schaefer *et al.*, (1974).
4 – 24 mg m^{-3}	Miner and Hazen, (1969).

*Adapted from O'Neill and Phillips (1992) from * down.*

Phillips *et al.* (1998), Groot Koerkamp *et al.* (1998) and Wathes *et al.* (1998) conducted their studies in collaboration with each other and examined the concentrations of ammonia in livestock farms in Britain, The Netherlands, Denmark and Germany. They found equal concentrations in all areas regardless of the geographical location. Ammonia emitted from agricultural activities can be transported over long distances and can be deposited into sensitive terrestrial and aquatic ecosystems (Schjorring, 1998). However, ammonia has a high deposition velocity and therefore the majority of emitted ammonia is deposited very close to the source (van der Eerden *et al.*, 1998), which results in the accumulation of the pollutant in a particular area over time. 80-95% of the total ammonia emissions in Europe has been estimated to originate from agricultural practices, the remainder is due to industrial sources, households, pet animals and natural ecosystems. Of the total ammonia emissions from livestock farming 80 % is from animal excreta and the remaining 20 % is from use of fertilisers. There is a large variation of ammonia emissions from country to country (van der Hoek, 1998). In 1995 ammonia emissions from The Netherlands was 181×10^{6} kg ammonia, 87 % of which was due to animal husbandry. The remaining was from the use of artificial fertiliser, industry

and household wastes (van der Eerden *et al.*, 1998). The Netherlands currently has the highest nitrogen deposition in Europe. Inventories on ammonia emissions in Canada showed similar trends with respect to sources of emissions as those in Europe. The 1990 inventory, found that ammonia emissions were 651 ktonnes and 87 % was attributed to agriculture (Kurvits and Marta, 1998).

1.1.3 Effects of ammonia pollution

Ammonia in solution at low temperature and pH values forms ammonium ion (NH_4^+), which is non-toxic to the organisms at medium concentrations. However, as the pH increases, the non-ionised form, ammonia (NH_3) is the dominant species, which is toxic to organisms even at low concentrations. Also as the pH increases ammonia can cross the cell membrane much more readily than at lower pHs (http://h2osparc.wq.ncsu.edu/info/ nh3.html 05-01-2001). Excesses of ammonia may accumulate in organisms thus causing alteration of metabolism or increases in body pH. Dry deposition of ammonia can affect plant metabolism at a cellular and whole plant level. Especially sensitive crops include arboriculture conifers and greenhouse crops such as tomatoes and cucumbers. The effects on the conifers are often caused by reduced frost tolerance as a result of a too high foliar N content relative to other nutrients. The effects on glasshouse plants includes severe disturbance of cellular pH, which causes reduced flowering and retarded fruit ripening (van der Eerden *et al.,* 1998). A North America study also showed effects of ammonia on animals. It was found that there was a reduction in weight gain by pigs in an environment that contained 50 ppm ammonia (Drummond *et al.,* 1980). Ammonia emissions are of interest not only for the deleterious affect on the environment, including acidification of soils and groundwaters and accelerated losses of biological diversity (Kurvits and Marta, 1998) but because of the odour associated with such emissions (Miner, 1977).

1.1.4 Regulations controlling gaseous emissions

Concern for the environmental effects of these nitrogenous emissions has led to considerable science and policy efforts, particularly in Europe, to determine the need and options for systematic and targeted management of the air emissions. Domestic and international initiatives, such as the Multi-pollutants, Multi-effects Protocol under the Convention on Long Range Transboundary of Air Pollution (LRTAP) of the United Nations Economic Commission for Europe (UN-ECE), are promoting

environmental management actions (Bull, 1991). The EC has adopted a proposal for a directive on national emission ceilings for certain atmospheric pollutants and a proposal for a directive relating to ozone in ambient air (Cowell and ApSimon, 1998). The National Emission Ceilings Directive will, for the first time, set individual limits for each member State's total emissions in 2010 of the four pollutants responsible for acidification, eutrophication and ozone formation, in the lower atmosphere: sulphur dioxide, nitrogen oxides, volatile organic compounds and ammonia. In implementing the Emission Ceilings Directive, Member States will need to assess what action is appropriate in their particular circumstances, and introduce measures accordingly (http://europa.eu.int/comm/environment/docum/99125sm.htm, 05-01-2001).

In Ireland, the EPA Act 1992 provided for an Integrated Pollution Control (IPC) licensing system to control industrial emissions. Associated with each industrial sector is a Best Available Technology Not Entailing Excessive Costs (BATNEEC) guidance note, which defines the emission standards that should be achieved by new plants. The effects of the emissions on the environment and the local community, including odour nuisance are accounted for in the guidance notes. Ammonia is included in those notes where it is a significant by-product of the industrial activity (http://www.epa.ie/licences/default.htm, 05-01-2001).

1.2 Air pollution control (APC) technologies

Limiting emissions into the atmosphere is both technically difficult and expensive. There are a number of means by which air pollutants from industrial sources can be controlled.

1. Modifying or eliminating the pollutant-generating processes in order to reduce the pollutant. Sometimes it is easier and less expensive to use alternative methods of production than to trap the effluent.
2. Collection and recycling of the effluent gas.
3. In some cases gases emitted from industrial sources are at very high temperatures, and on cooling they are converted to liquid form, which is easier to collect and handle. Gas cooling can be achieved by heat exchange and therefore thermal energy can be recycled back into the process.

4. The pollutants may be removed from the effluent stream by trapping them from the stream or by changing them chemically or biologically (Vesilind *et al.*, 1994).

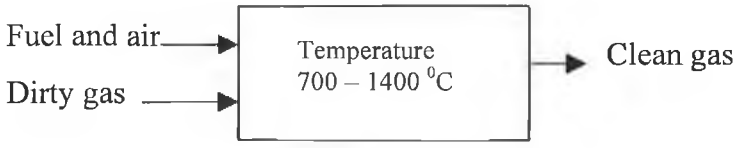
Traditionally physical or chemical techniques including incineration, wet chemical scrubbing, condensation, chemical additions and adsorption were used to remove pollutants from waste gas streams.

1.2.1 Incineration

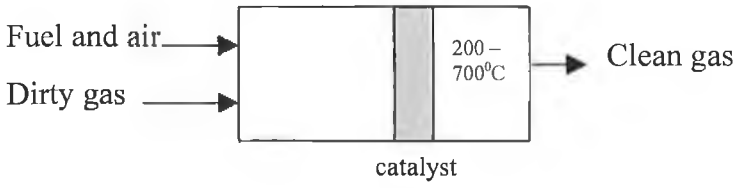
Incineration involves oxidation of the waste gas to CO_2 and water by heating the waste gas at high temperatures e.g. 700 - 1400 ^{0}C (Vesilind *et al.*, 1994 and Deshusses, 1997). Incineration of waste gas streams can be achieved thermically, catalytically or electrically. The use of catalysts in the incineration process allows for lower temperatures of operation (200 – 700 ^{0}C). Electrical incineration involves the incineration of the gases in the presence of an electric field, which allows operation at lower temperatures and at inlet oxygen concentrations as low as 4 % (Deshusses, 1994)

Fig. 1 Comparison between thermal incineration and catalytic incineration (adapted from Vesilind *et al.*, 1994)

Thermal Incineration



Catalytic incineration



1.2.2 Wet chemical scrubbing

This process involves dissolving the pollutant of concern in a scrubber solution. SO_2 and NO_2 from power plant exhaust gases are often treated in this way (Vesilind *et al.,* 1994). The pollutants are transferred from the gaseous phase to the liquid phase, and then have to be further treated. In some cases the pollutant is absorbed so that a reuseable product is created (Schifftner and Hesketh, 1986).

1.2.3 Adsorption

The pollutant is adsorbed to a chemical adsorbent such as activated carbon in a fixed or fluidised bed. This method is primarily used for the treatment of organic compounds (Vesilind *et al.*, 1994). Once the adsorbance capacity of the bed is reached, the pollutant must be de-sorbed in order to regenerate the material. Alternatively the adsorbing material together with the adsorbed pollutant must be incinerated (Deshusses, 1994).

1.2.4 Condensation

This technique involves the simultaneous cooling and compressing of gaseous pollutants. The method is generally employed when there are high concentrations of the pollutant in the gas stream. However, further treatment of the pollutant is usually required, prior to discharge of the waste stream, in order to reduce the concentration of the pollutant to legally accepted levels (Deshusses, 1994).

1.3 Biotechniques for air pollution control

Biological methods are also employed to treat waste gas streams. The use of biological methods is a much more recent phenomenon than the use of chemical and physical methods, although biofiltration has been used periodically since the 1920's (Kennes and Thalasso, 1998). Biological methods offer many advantages over the other methods. Biological methods tend to have lower installation, operational and maintenance charges than chemical methods and they are operated at ambient temperature and atmospheric pressure and the pollutant is not merely transferred from the gaseous phase into a liquid or solid phase as is the case with physical techniques (Ottengraf, 1986 and Utkin *et al.*, 1990). In biological methods micro-

organisms are exploited for their ability to utilise compounds, that are generally regarded as pollutants, as an energy source (Ottengraf, 1986). Hydrophilic pollutants are more suited to biological treatments because biological methods involve the sorption of the pollutant into a liquid (water) phase (Utkin *et al.,* 1990 and Devinny, 1998). Biological techniques are also more easily applied if the pollutant to be treated is present at low concentrations in the waste gas stream (Devinny, 1998). Biological air pollution control techniques include bioscrubbing, trickling filters and biofilters. The methods can be distinguished from each other by the behaviour of the liquid phase and of the micro-organisms, which can be freely dispersed in the aqueous phase or immobilised on the packing material.

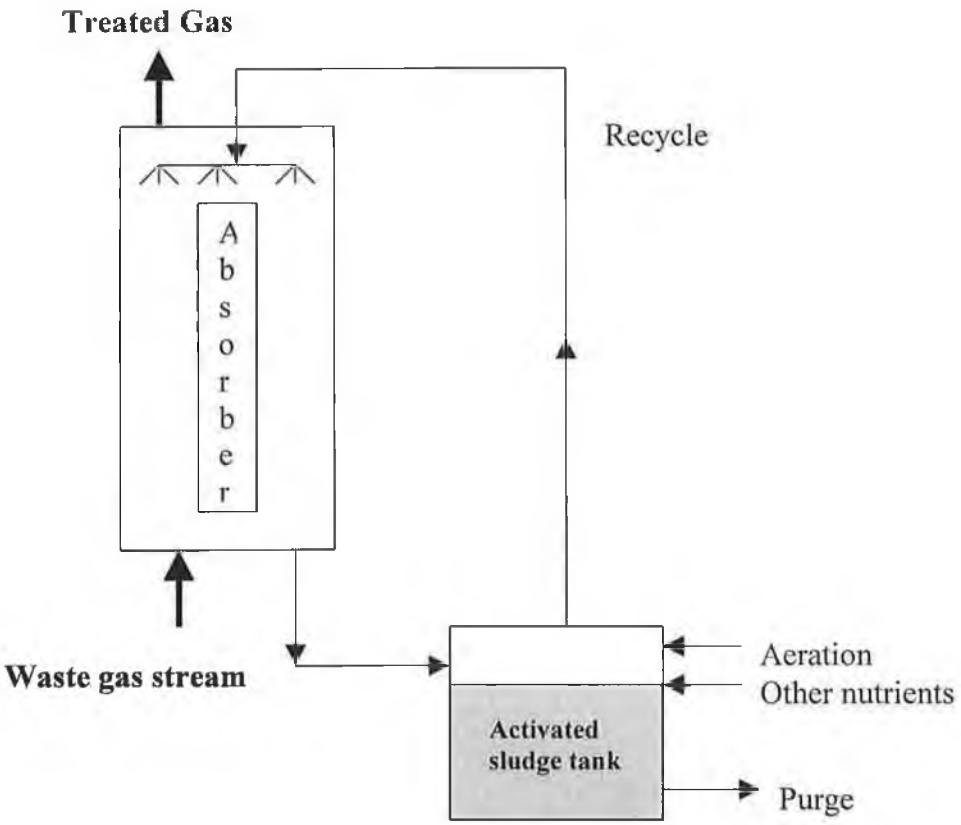
Table 2 Distinctions between biological waste gas purification systems *(adapted from Ottengraf, 1987)*

	Aqueous phase	**Microbial flora**
Bioscrubber	Mobile	Dispersed
Trickling filter	Mobile	Immobilised
Biofilter	Stationary	Immobilised

1.3.1 Bioscrubbers (Fig. 2)

The pollutant is contacted with a liquid phase (water) in a reactor such as a spray column. Water soluble components absorb from the gas stream into the liquid phase on contact. The absorbed components (pollutant of interest) are transported in the mobile liquid phase to a separate activated sludge tank where biodegradation occurs (Kennes and Thalasso, 1998). The microbial populations are freely dispersed in the liquid phase. Essential nutrients, required for the growth of the organisms, are supplied in the liquid phase together with oxygen required for aerobic degradation of the pollutant.

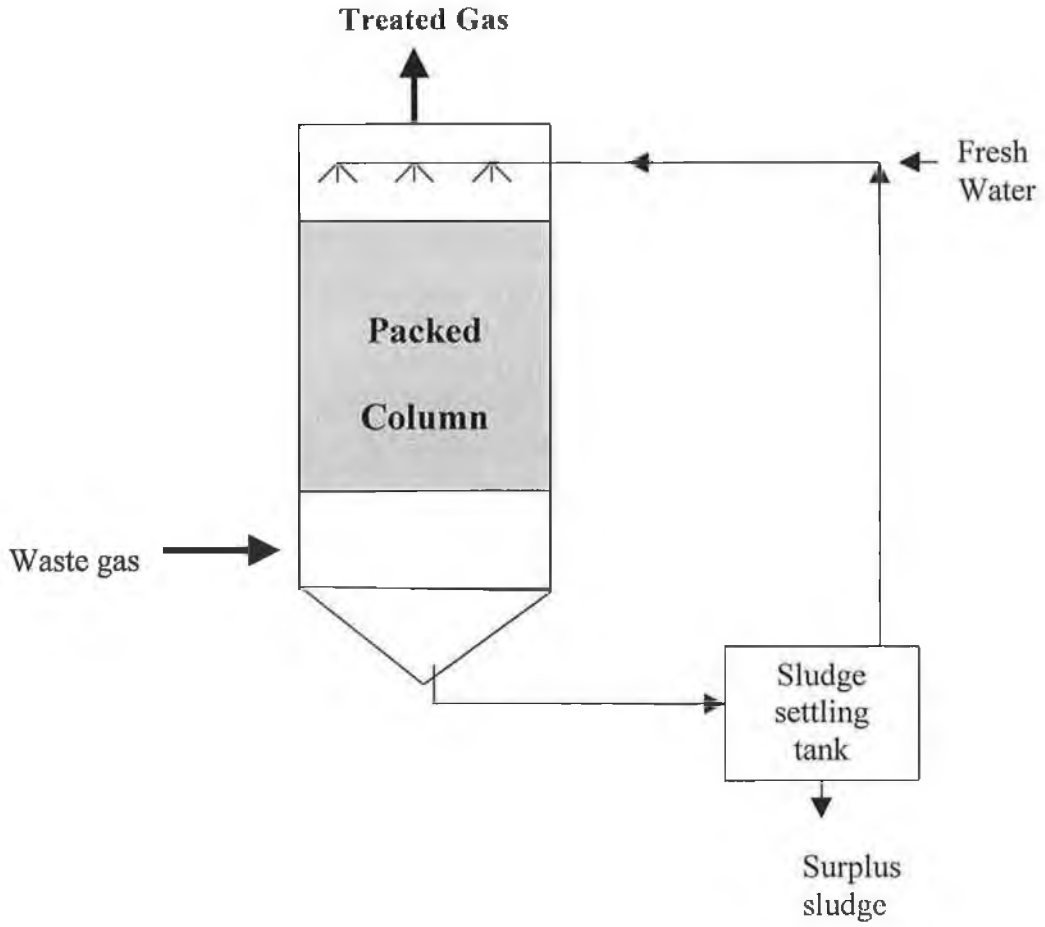
Fig. 2 Bioscrubbers



1.3.2 Trickling filters (Fig. 3)

Trickling filters operate on the same principles as bioscrubbers only the micro-organisms are immobilised on a packing material and both absorption and biodegradation occur in the same vessel.

Fig. 3 Trickling Filter



1.3.3 Biofilters

Biofilters utilise an immobilised microbial population for the degradation of pollutants, in the same way as trickling filters however there is no mobile liquid phase. Both adsorption and degradation occur in the same vessel. Although there is no mobile liquid phase water is regularly added directly to the filter bed to ensure adequate moisture levels for optimal microbial activity and to add essential nutrients. A more detailed description of biofiltration is given in Section 1.4. Of the three biotechniques described for air pollution control biofilters are more suitable for the degradation of the poorly water-soluble pollutants because there is no mobile liquid phase (Kennes and Thalasso, 1998).

1.4 Biofiltration – Process overview

1.4.1 History of biofilters

Biofiltration is the oldest biotechnological method for the treatment of waste gases and it originated in Europe for odour abatement. Traditionally it was used for the treatment of off-gases from wastewater treatment facilities and has since developed into a sophisticated method for the removal of volatile organic compounds (van Groenestijn and Hesselink, 1993).

Even back as early as 1923 the basic concept of control of hydrogen sulphide emissions from sewage treatment plants was discussed and although one of the earliest patents in the field was applied in 1934, successful biofilter applications were not reported until the 1950's (Leson and Winer, 1991 and Ottengraf and Diks, 1991). Pomeroy received U.S. patent in 1957 for his installation of a soil bed in California. One of the first reported applications of biofiltration in Europe was in 1959 when a soil bed was installed for the treatment of gases emitted from a municipal sewage treatment works in Nuremberg, W. Germany (Leson and winer, 1991).

Carlson and Leiser carried out the first systematic research on hydrogen sulphide treatment using biofiltration in the 1960's. Application and research on soil beds found that the beds are prone to clogging and drying out, therefore alternative bed materials were examined and the use of compost based materials derived from municipal solid waste has been used since 1966 (Leson and Winer, 1991). By the 1970's more advanced open biofilters with air distribution/media support system had been developed with most of the development work being carried out in The Netherlands and Germany where more stringent national regulatory requirements on gaseous emissions had been implemented. Even with the new advanced biofiltration systems common problems included drying-out of the filter bed, rapid media compaction and acidification by degradation products. This stimulated further research in the 1980's and resulted in the development of fully enclosed systems using more porous media (van Lith *et al.,* 1997). Since then biofiltration has become increasingly popular in Europe, particularly in Germany for the treatment of volatile organic compounds and hazardous air pollutants from chemical plants and coating operations. The use of biofilters was slower to be accepted in America compared to Europe. This is due to the fact that there was a lack of process descriptions written in the English language and there was little governmental support for research in the

field (Leson and Winer, 1991). However amendments to the Clean Air Act in 1990 stimulated American interest and research into biofiltration as a waste gas treatment technology (Swanson and Loehr, 1997).

1.4.2 Biofilter design and construction

A biofilter consists of one or more beds of biologically active material through which waste gases are vented. There are five major components involved in any biofiltration unit

i. A ventilator, which is responsible for the flowrate and the pressure drop across the filter bed. In closed systems the ventilator can be placed before the humidifier as is the case for up-flow biofilters or at the end of the process as an extractor fan in the case of down-flow biofilters. The ventilator is usually the most costly component of the system.
ii. A humidifier, which is required to increase the relative to humidity of the inlet gas. If the inlet air stream is not saturated, it will strip moisture from the biofilter bed as is passes through.
iii. A waste gas distribution system to ensure even distribution of the inlet gas through the packed bed. Inhomogeneous distribution of the gas stream leads to channelling and therefore inadequate contact times.
iv. One or more layers of filter material, which serves to immobilise the microbial population and also acts as a moisture and nutrient reservoir.
v. An additional water supply, to ensure adequate moisture within the biofilter bed. Moisture may be stripped from the biofilter bed as a result of inadequate humidity of the inlet air or due to exothermic reactions such as biooxidation within the biofilter bed material (Deshusses, 1994).

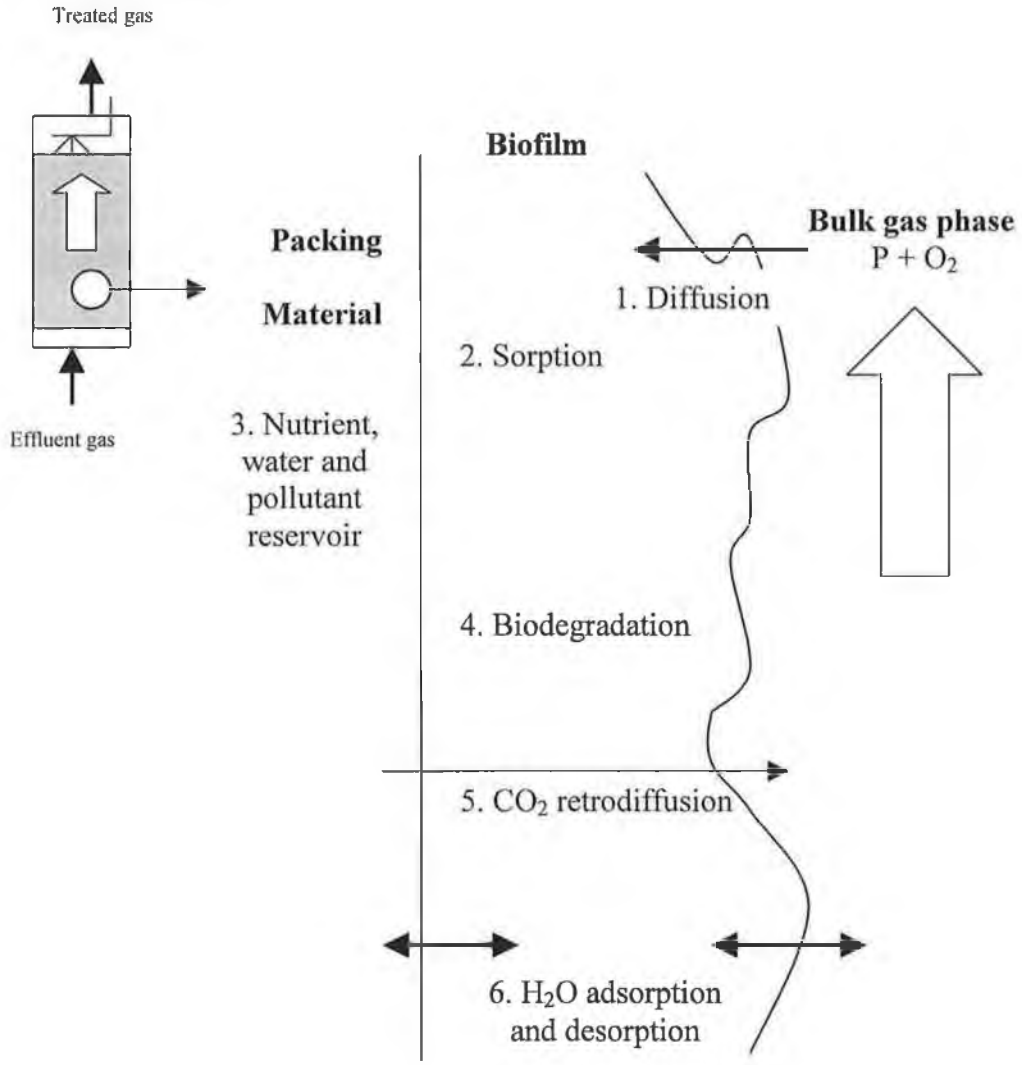
Biofilters may be constructed as opened or closed systems, they can be operated in an up-flow or down-flow manner and they can be single or multi- layer (Swanson and Loehr, 1997, Toffey, 1997 and Utkin *et al.,* 1990). Traditionally full-scale biofilters were built as open structures, however there are drawbacks associated with these structures as they are subjected to all weather conditions (Leson and Winer, 1991). Extreme heat and cold affects the growth and metabolism of the micro-organisms, precipitation levels alters the moisture content of the bed and harsh conditions can damage the structure of the packing material and cause channelling of the air. In recent years emphasis has been placed on the development of closed

systems, which allows for better control. Multi-storey biofilters are generally constructed when space constraints arise.

1.4.3 Theory of operation

Basically biofiltration involves the aerobic conversion of a pollutant by an immobilised microbial population. The immobilised micro-organisms form a liquid biolayer in the moisture surrounding the packing material (biofilm) in which the biodegradation occurs. The macrokinetics of pollutant elimination in a packed bed involves a series of mass transfers and diffusions, which can be described as pollutant absorption into the biofilm followed by simultaneous oxidation by the intrinsic micro-organisms, which are outlined below (Fig. 4) (Deshusses, 1997, Leson and Winer, 1991 and van Lith *et al.*, 1997).

Fig. 4 Macrokinetics of biofiltration

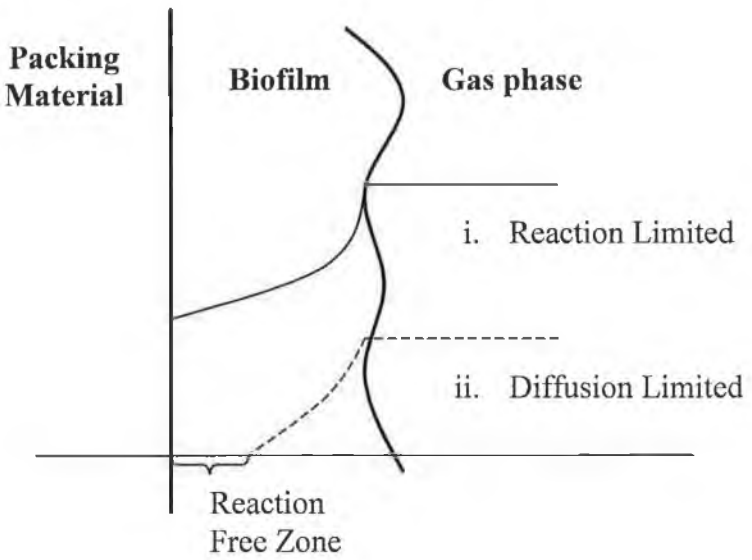


(adapted from Deshusses, 1994)

1. The pollutants (P) and oxygen diffuse from the bulk gas phase into the liquid biofilm surrounding the packing material.
2. Parts of the pollutants sorb onto the packing material itself.
3. The packing material acts as a reservoir for water, nutrients and pollutants, which can easily sorb back into the biofilm.
4. The pollutants are degraded by the immobilised micro-organisms in the biofilm.
5. End products e.g. CO_2 diffuse back into the bulk gas phase and are transported out of the biofilter.
6. Water is continually adsorbed and desorbed by the packing material.

7. The continuous degradation of pollutants in the liquid phase results in a concentration gradient in the biofilm, which allows continual absorption of the target pollutants in the biofilm. During biofiltration the rate of reaction within the biofilm is either substrate limited or diffusion limited, which gives rise to the concentration profiles depicted in Fig. 5 (Ottengraf, 1986).

Fig. 5 Pollutant concentration profile within a biofilter



(Adapted from Ottengraf, 1986)

i. During reaction limitations the biofilm is fully active and there are no diffusion limitations.
ii. At low substrate concentrations the system is usually diffusion limited. There is a reaction-free zone in the biofilm, close to the packing material, in which no degradation occurs.

Theoretical models describing biofiltration processes have been derived based on these substrate concentration profiles within the biofilm. However as there is still a lack of information on the quantification of biomass turnover, on the complex ecology of the microflora and on the determination of the cycles of the pollutant, oxygen and essential nutrients within the biofilter a universally acceptable model has not been developed. Unfortunately current knowledge of biofiltration is essentially empirical and biofilters are still considered as "black boxes". Many biofilters have

been scaled-up on an empirical basis, which has led sub-optimal operation and much distrust by industries on the use of biofilters (Deshusses, 1994).

1.4.4 Process design

Successful operation of a biofiltration system requires knowledge and understanding of the physical and biological parameters involved, including air and contaminant load, medium characteristics, bed permeability, water content and biological activity (Devinny, 1998). Any systems involving biofilms are complex due to a combination of factors, such as bacterial growth, substrate consumption, attachment, external-internal mass transfer of substrate and products, cell death, shear loss, sloughing (fragments disrupting from the film), competition between bacterial species, and the effects of predators. All of these factors play a significant role in the overall capacity of the biofilm process and are either directly or indirectly affected by the biological and physical parameters (Wijffels & Tramper, 1995).

Packing materials

The packing material is the key component of any biofiltration system. It must provide an optimal environment for the growth of the immobilised micro-organisms, it must also be capable of maintaining its physical integrity and provide a flow resistance such that the residence time is optimal (Leson and Winer, 1991). Many different types of materials have been employed in biofiltration units, which are chosen on the basis of their physical and mechanical properties. Ideally the bed material should provide

- a large enough surface area to maximise contact between the pollutant and the microbial population
- a particle size that gives an acceptable flow resistance
- a high void fraction to prevent pore clogging problems
- low levels of compaction
- a reasonable adsorbing surface to minimise shock loading problems
- a capacity to buffer acidification due to metabolic end products.

Examples of previously researched organic packing materials together with advantages and disadvantages associated with their use in biofiltration systems are

outlined below (Table 3) (Bohn, 1992, Kennes and Thalasso, 1998, Leson and Winer, 1991, Swanson and Loehr, 1997 and Wani *et al.,* 1997).

Table 3 Advantages and disadvantages of organic packing materials

Media Type	Advantages	Disadvantages
Soil	Cheap	Compacts easily, causing an increase in pressure drop thus increasing energy requirements and reducing efficiency
	Readily available	Low air permeability
	Safe	Large area required
	Low maintenance requirements	
	Large microbial population	
	Unlimited lifetime	
Compost	Large surface area	Higher maintenance requirements than soil
	High air permeability	Ageing due to mineralisation
	High water permeability & holding capacity	Settling results in short-circuiting
	Large microbial population (greater than soil)	Must be changed every 2-5 years
Peat	Good adsorption-absorption properties	Ageing due to mineralisation
	High cellulose content	Compaction
	Cheap and easily available	Must be changed every 2-5 years
	Buffering capacity	
	High moisture retention capacity	

Other commonly used bed materials include heather, bark, leaves and wood chips, which are often mixed with peat or compost to improve the permeability and structural integrity of the support matrix (Swanson and Loehr, 1997 and van Langenhove *et al.*, 1988). A disadvantage associated with all organic packing materials is that they need to be stirred regularly to prevent cracking. They are also subject to compaction, which can be minimised by mixing inorganic, inert materials such as granular activated carbon, rubber, perlite and polystyrene beads with the organic material (Wani *et al.,* 1997). Granular activated carbon also serves to minimise shock-loading problems due to its higher adsorptive capacity. Inorganic, inert materials have also been used on their own as packing materials, however they are more expensive than organic materials and maintenance requirements are higher in that additional nutrient must be supplied to the immobilised micro-organisms (Swanson and Loehr, 1997). The addition of basic solids or alkaline materials in the irrigation water e.g. calcium hydroxide have been used to improve the buffering capacity of some packing materials (Wani *et al.* 1997).

Gas supply

The removal efficiency of a biofilter is governed by the residence time i.e. the length of time the pollutant is in contact with the microbial biomass and the contaminant load. Generally the residence time is indirectly measured as the empty bed contact time (EBCT), which is a function of the gas flowrate (Devinny, 1998). High flowrates results in shorter EBCT and therefore lower removal efficiencies. High contaminant loads can be a result of high concentrations or high flowrates both of which lead to a decrease in efficiency (Swanson and Loehr, 1997). The permeability of the bed also has a role to play in the residence time of a pollutant within a filter bed. Gas streams will always flow along the path of least resistance e.g. along the walls of the biofilter or through cracks that are caused by compaction or drying of the bed, which leads to reduced EBCT and therefore the emergence of partially treated gases (Devinny, 1998).

On the other hand excessively long empty bed contact times implies that the biofilter is not operating optimally. Long empty bed contact times occurs when the bed permeability decreases. Bed permeability is reduced if grease and resins in the inlet air stream, smaller bed particles or excessive biomass growth clogs the pores (Leson and Winer, 1991). A reduction in bed permeability also leads to increased pressure

drop across the packed bed and therefore leads to higher energy consumption (Devinny, 1998). In order to prevent pore clogging dust particles and fat should be removed from the inlet gas stream prior to entering the biofilter. Excessive biomass growth can be the result of the supply of high loads or the supplementation of biofilters with additional nutrients. Martin et al., (1996) found that there was an increase in the heterotrophic population when additional nutrients were supplied to the biofilter.

Microbial environment

As with all microbial systems, an energy source, adequate moisture, mineral nutrients, oxygen and a temperature of 5 – 50 ^{0}C are required by the micro-organisms involved in biofiltration. All five growth conditions are necessary and affect the reaction kinetics, however the moisture level within the packed bed is the most important control parameter (Bohn, 1993). In most biological reactors water is in excess and easily manipulated but in biofilters the packing material is not saturated and there is no mobile liquid phase (Ottengraf, 1986). It is also very difficult to determine and adjust the water content of a biofilter. Excessive moisture leads to anaerobic regions in the bed, diffusion limitations, slime layer formation and clogging of pores, which results in increased pressure drops. Insufficient moisture causes a decrease in microbial activity and it also causes the packing material to dry and therefore shrink and crack, which results in by-pass flow and a reduction in the overall performance (Swanson and Loehr, 1997). It also leads to accelerated fungal growth. Air and water compete for pore spaces and at proper moisture levels the air flows through the larger pores and the water is restricted to the smaller pores. The aim of moisture control is to ensure that fluctuations in the moisture of the filter bed are not so large that the efficiency of the system is significantly affected. van Lith *et al*, 1997 reported that in some instances the moisture level in biofilters varied by as much as 30 L m^{-3} packing material from the optimal level without any significant changes in the overall performance. For most biofilter applications the biofilter bed should be maintained at a neutral pH as micro-organisms generally prefer a neutral environment. The packing material should provide adequate buffering capacity especially if acidic metabolites are produced as a result of microbial degradation (Kennes and Thalasso, 1998). In some cases it is necessary to pre-treat the packing material with neutralising agents, however the addition of powdered substances may

lead to pore clogging. For some microbial populations essential nutrients may be required as a separate addition to the system, however as already stated additional nutrients should be kept minimal to prevent excessive biomass production (Devinny, 1998). As most micro-organisms used in biofiltration are aerobic, it is required that a minimum of 100 parts oxygen to every part of oxidisable gas be supplied to ensure sufficient levels of oxygen. Oxygen limitation is generally not a problem as oxygen is usually in abundance in the inlet air. If the system is overloaded with high concentrations of the pollutant, oxygen limitations may arise and it often results in formation and accumulation of acidic and other metabolic intermediates (Wani *et al.*, 1997).

1.4.5 Biofilter uses

Biofilters have been used to treat a variety of organic and inorganic pollutants in industrial and municipal exhaust streams. Among those are odorous gases (ammonia, hydrogen sulphide and mercaptans), food processing wastes, gases from waste water treatment plants and composting facilities and VOC from industries (Wani *et al.*, 1997). However not all pollutants are equally suited to biofiltration. The degradability of some organic and inorganic pollutant by are listed below (Table 4).

Table 4 Degradability of compounds in biofilters *(Adapted from Deshusses, 1994)*

++ excellent degradability

+ good degradability

(+) minimum degradability

- no degradability

? no certain knowledge

ORGANIC COMPOUNDS			
Oxygen containing compounds		**Aliphatic hydrocarbons**	
- alcohols	++	- saturated aliphatic hydrocarbons	
methanol	++	methane	(+)
butanol	++	pentane	(+)
- ethers	(+)	hexane	+
tetrahydrofurane	++	- unsaturated aliphatic hydrocarbons	

Table 4 Degradability of compounds in biofilters (ctnd.)

diethylether	(+)	acetylene	?
dioxane	(+)	- cyclic aliphatic hydrocarbons	
- aldehydes	++	cyclohexane	(+)
formaldehyde	++	**Aromatic hydrcarbons**	
acetylaldehyde	++	benzene	+
- ketones	+	toluene	++
acetone	+	xylene	++
- carbonic acids	++	styrene	+
butyric acid	++	**Halogenated hydrocarbons**	
- esters of carbonic acids	+	dichloromethane	(+)
ethyl acetate	+	trichloroethylene	?
methyl methacrylate	?	perchloroethylene	?
-phenols	+	chlorophenols	+
Sulphur containing compounds		1,1,1 - trichloroethane	-
- sulphides (thioether)	+	**Nitrogen containing compounds**	
Dimethyl sulphide	+	- amides	+
- thiocyanates	+	- amines	++
-isothiocyanates	?	trimethylamine	++
- sulphur heterocycles	+	- nitrogen heterocycles	+
thiophene	+	pyridine	+
- mercaptans	+	- isocyanates	?
methyl mercaptan	+	- nitro compounds	(+)
- carbon disulphide	+	- nitriles	+
		acetonitrile	+
		- isonitriles	+
INORGANIC COMPOUNDS			
hydrogen sulphide	+		
ammonia	+		

Biofilters can also be used to treat waste gases containing mixtures but there are competitive effects between chemicals in both mass transfer and degradation that can lead to accumulation of one compound and may lead to toxicity effects (Swanson

and Loehr, 1997). According to Bohn (1993) there are more than 25 million small biofilters in operation in North America as part of household septic tanks and there are hundreds of full-scale biofilters operating world-wide. Examples of full-scale biofilters are given in Table 5, which shows the wide variety of components successfully treated by biofilters and also gives an indication of the diversity in terms biofilter sizes, flowrates etc that can be applied to biofilter design.

Table 5 Examples of full-scale operational biofilters *(adapted from Deshusses, 1994)*

Industry	Packed vol. (m^3)	Packing material	Moisture (%)	Temp. (^0C)	Flowrate ($m^3\ h^{-1}$)	Gas composition	Inlet conc. ($mg\ m^{-3}$)	Removal efficiency (%)
Rayon production	13	bark & compost	65	25 - 30	2925	H_2S CS_2	80 140	88 57
Foundry	300	heather & peat	n.i.	n.i.	n.i.	total carbon benzene	130 13	69 85
Sewage sludge composting	1150	heather & peat	60	20	98000	odour	9000 OU m^{-3}	83
Manure drying	200	heather	n.i.	35	20000	1. VOC's 2. organically bound carbon 3. aldehydes 4.organic acids 5. ammonia 6. odour	342 172 9000 9 6000 0.31 OU m^{-3}	51 47 85 95 75 82

Note: All of the biofilter beds had a packed height of 1 m. The biofilter treating the air from the foundry was a multi-layer system.

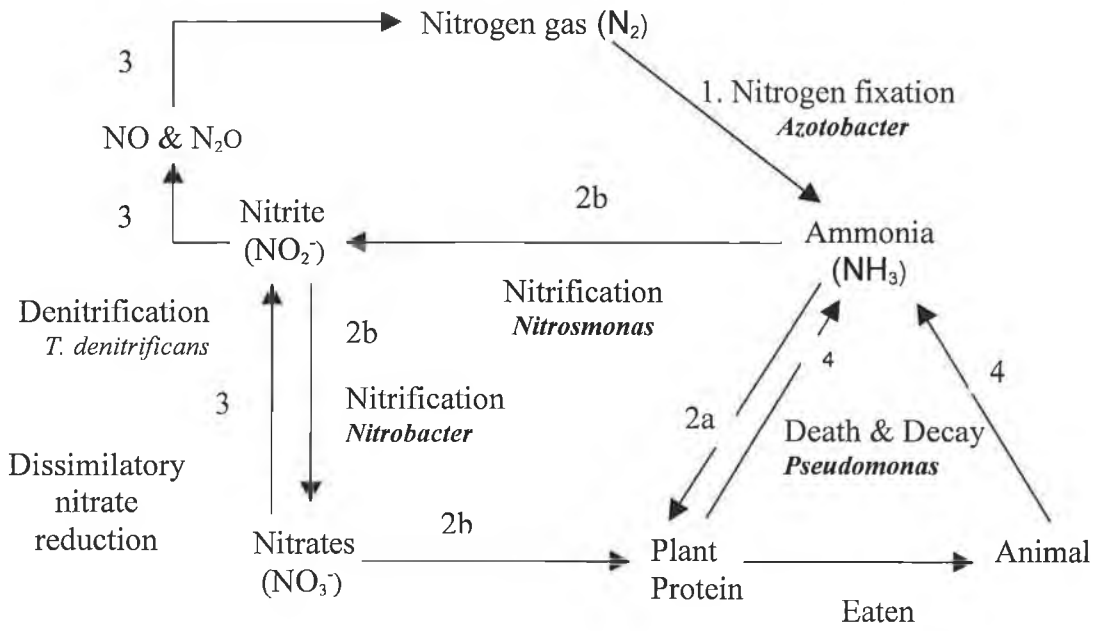
n.i. = no information given OU = odour units

1.5 Microbiology

1.5.1 The nitrogen cycle

Nitrogen is required by all organisms for the metabolism of amino acids and nucleotides. The atmosphere, the most abundant source of nitrogen, comprises approximately 79% nitrogen (http://www.bact.wisc.edu/microtextbook/Metabolism/NitrogenAssim.html 5-01-2001). Nitrogen is also found as organic matter in soil and the ocean. Although it is very abundant it is commonly the limiting nutrient for plant growth because plants can not use it in its gaseous form. Plants can only take up nitrogen in two solid forms (i) as ammonium ion, (NH_4^+) or as (ii) nitrate ion, (NO_3^-). High concentrations of ammonium are toxic to most plants and therefore the uptake of nitrate is the preferred method of nitrogen assimilation (http://www.geog.ouc.bc.ca/physgeog/contents/9s.html 05-01-2001).

Fig. 6 Schematic of the Nitrogen Cycle



1. Nitrogen fixation is the conversion of nitrogen gas to ammonia, (NH_3); this dissolves to become ammonium, (NH_4^+). Lightening, volcanic action and many microorganisms e.g. Rhizobium, Clostridium and Azotobacter fix atmospheric nitrogen. The process involves symbiotic and non-symbiotic organisms.

2. Ammonium has two fates

    (a) the plants assimilate and utilise the fixed nitrogen in the form of ammonium ion for the biosynthesis of organic amino acids, proteins and nucleic acids or

    (b) it is converted to the less toxic form, nitrate by microorganisms in a process called nitrification and is then assimilated by the plants.

Animals consume the plants and the nitrogenous compounds are converted to animal proteins. This is the only source of nitrogen for animals.

3. Nitrate may also be denitrified, a process whereby nitrates are reduced to nitrogen gas and released back into the atmosphere.

4. Excretion products of animals, dead animals, plant tissues and microorganisms deposit organic nitrogen on the soil and it is further degraded by microorganisms to release ammonia by a deamination process. The re-release of ammonia back into the soil is termed ammonification (http://www.geog.ouc.bc.ca/physgeog/contents/9s.html and http://clab.cecil.cc.md.us/faculty/biology/jason/nitrc.htm 05-01-2001).

In this way nitrogen is cycled through the biosphere.

Nitrification

Nitrification is the oxidation of ammonia to nitrate by microbial activity. It is a two step aerobic process (Boch *et al.*, 1991).

Oxidation of ammonia to nitrite by ammonia-oxidising bacteria

$$NH_3 + ½\ O_2 \rightarrow NH_2OH$$

$$NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^-$$

Where NH_2OH, hydroxylamine is the intermediary in ammonia oxidation.

Oxidation of nitrite to nitrate by nitrite-oxidising bacteria

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$$

$$2H^+ + 2e^- + \tfrac{1}{2} O_2 \rightarrow H_2O$$

Denitrification

Denitrification is the production of gaseous nitrogen by microbial reduction of nitrogenous compounds. The substrates usually used by the organisms are nitrites or nitrates and the products are di-nitrogen gas (N_2), nitrous oxide (N_2O) and nitric oxide (NO). Nitrous oxide is an intermediate in most cases of denitrification but the occurrence of nitric oxide is less frequent. Denitrification is an anaerobic process that is generally carried out by heterotrophic, facultative aerobes. In the absence of oxygen they utilise nitrate as the terminal electron carrier. There are also some autotrophic nitrifiers e.g. *Paracoccus denitrificans* and *Thiobacillus* (Tiedje, 1982).

1.5.2 Nitrifying organisms

Autotrophic Nitrifiers

The nitrifying bacteria are members of the family *Nitrobacteracaea* and it consists of two groups (i) the ammonia-oxidisers and (ii) the nitrite-oxidisers. All members of the family are obligate autolithotrophs with the exception of some *Nitrobacters,* which are capable of growing heterotrophically but it is a very inefficient process. Some autotrophic nitrifiers can assimilate organic carbon to a limited extent during mixotrophic growth also however mixotrophic growth is less efficient than pure autotrophic growth (Watson *et al.*, 1989). Stuven *et al.,* (1992) found that under aerobic conditions in autotrophic media that all the ammonia, (142 mg N L^{-1}) was converted to nitrite by Nitrosomonas cells but when the cells were grown on mixotrophic media there was a loss of 8 +/- 3% of total inorganic nitrogen. Approximately 121 mg N L^{-1} was converted to nitrite and approximately 12 mg L^{-1} either remained in the media as NH_4^+ or was assimilated directly into the cell for growth.

Autotrophic bacteria are very slow growing organisms that obtain energy for growth by oxidising ammonia or nitrite and they utilise carbon dioxide as the main carbon source. Although they are slow growing they are very efficient ammonia and nitrite

oxidisers. According to Allison and Prosser, (1992) an ammonia-oxidiser must convert 33% of its own mass of ammonia per hour to double in size. The rates of product formation for ammonia and nitrite oxidation are 1000 - 30,000 mg N d^{-1} g^{-1} dry weight cells and 5000 - 70000 mg N d^{-1} g^{-1} dry weight cells respectively (http://www.bsi.vt.edu/chagedor/biol_4684 /Cycles/Nitrification.html 05-01-2001).

Nitrifiers grow optimally at a temperature of 25 – 30^0C at pH 7.5 – 8.0 and a substrate concentration of 5 – 50 mM for ammonia-oxidisers and 2 – 30 mM for nitrite-oxidisers (Boch *et al.*, 1991). However Watson *et al.* (1989) reported that optimal growth occurs at a substrate concentration of 2 – 10 mM ammonium.

Nitrification limitation in soils is usually as a result of non-optimal ammonia, carbon dioxide or oxygen concentrations or environmental conditions such as temperature and pH. The bacteria are also inhibited by non-polar organics e.g. hydrocarbons and alcohols.

Ammonia-oxidisers

Ammonia oxidisers are gram-negative bacteria and their morphology varies from genus to genus (Table 6).

Table 6 Ammonia-oxidisers *(adapted from Watson et al. 1989).*

Genus	Morphology	Growth Range	Habitat
Nitrosomonas	single rods	5 –30^0C, pH 5.8 – 8.5	soil, marine and freshwater
Nitrosococcus	spherical in pairs	5 – 30^0C, pH 6.0 – 8.0	soil, marine and freshwater
Nitrosospira	spiral	25 – 30^0C, pH 7.5 – 8.0	Soil and freshwater
Nitrosobolus	lobular, pleomorphic	13 – 30^0C, pH 6.0 – 8.2	soil
Nitrosovibrio	curved rods	-	freshwater

Free-living organisms have been found in low pH, low temperature and even hot temperature environments although there optimal growth temperature is 25 – 30^{0}C and optimal pH is pH 7.5 – 8.0 (Boch *et al.*, 1991). Natural strains can also survive in air dried soil for more than three months whereas laboratory strains, which were found to be missing significant amounts of extracellular polymeric substances as capsular material that is present in natural strains, could not survive more than 10 weeks (Allison and Prosser, 1991). Accumulation of nitrite, the end product of ammonia oxidation, inhibits the activity of the organisms. They are also sensitive to both UV and visible light (Boch *et al.*, 1991).

Nitrite-oxidisers

The nitrite oxidisers, although in the same family, *Nitrobacteracaea,* as the ammonia oxidisers, they are not phylogenically related. They are gram-negative bacteria. See Table 7 for nitrite-oxidising genera of *Nitrobacteracae*.

Table 7 Nitrite-oxidisers *(adapted from Watson et al., 1989)*

Genus	Morphology	Growth Range	Habitat
Nitrobacter	short rods	5 – 40^{0}C, pH 5.7 – 10.2	soil, marine, freshwater.
Nitrococcus	spherical	15 – 30^{0}C, pH 6.8 – 8.0	marine
Nitrospina	long slender rods	20 – 30^{0}C, pH 7.0 – 8.0	marine
Nitrospira	helical	-	soil, marine

In nature the nitrite-oxidisers have a much wider growth range than they have in pure culture. They have a generation time of 10 h to several days. They grow optimally at a temperature of 28 – 30^{0}C and a pH of 7.6 – 7.8 (Boch *et al.*, 1991). Heterotrophically growing *Nitrobacter* cells have an even longer generation time.

High oxygen partial pressures are inhibitory to *Nitrobacter* and under anaeorobic conditions they reduce nitrates to nitrites i.e. nitrite oxidation is a reversible process.

High ammonia concentrations can also inhibit *Nitrobacter* activity. Nitrate inhibits *Nitrobacter winogradski* at a concentration of 65 mM (Boch *et al.*, 1991).

Heterotrophic nitrifiers

Heterotrophic bacteria are also capable of nitrification but they do not appear to gain any energy for growth (Boch *et al.*, 1991). A wide range of heterotrophic organisms including bacteria, fungi and actinomycetes are capable of nitrification. Originally it was thought that all nitrification in soil was as a result of autotrophic organisms alone but in soil environments that are sub-optimal for autotrophic nitrification e.g. acidic soil, often it is heterotrophs that are responsible for the nitrification. Fungi play a significant role in heterotrophic ammonia oxidation. Their activities are generally lower than those of autotrophs and the process is not necessary for growth. The rates of formation are 0.012 to 1.70 mg N d^{-1} g^{-1} dry weight of cells. The importance of heterotrophic nitrification to the organisms is unclear; it is thought that the compounds produced may be antibiotic or growth factors (Kuenen *et al.*, 1988).

A study, which investigated the ability of heterotrophs to convert nitrite to nitrate, it was found that 17/48 produced almost as much nitrate as nitrite consumed, most of them were not capable of denitrification e.g. *Arthrobacter* and *Bacillus*. 12/48 did not convert all the nitrite consumed into nitrate but they were denitrification positive organisms e.g. *Corynebacterium* and *Pseudomonas*. 14/48 consumed nitrite but no nitrate was formed and 5/48 didn't utilise nitrite at all (Sakai *et al.*, 1996).

1.5.3 Nitrification limitations

Nitrifying organisms must be very resilient in order to survive in natural environments because they are such a slow growing group and they have to compete against the faster growing heterotophic organisms for limiting supplies of ammonia and oxygen. In most environments the concentrations of ammonia or oxygen are not constant and the organisms must have a way of dealing with such fluctuations. Due to the slow growing nature of autotrophs they are usually the disadvantaged group when it comes to limitations but they do survive in natural environments. Long-term survival in the absence of ammonium will be dependent on the ability to maintain large population sizes at the expense of endogenous energy sources and on the

preservation of a relatively large capacity for ammonium oxidation. In most natural ecosystems ammonia-oxidising bacteria are more likely to be limited by ammonium than by oxygen and oxygen consumption rates are lower under conditions of ammonium starvation (Gerards *et al.*, 1998). Jensen *et al.* (1993) studied nitrification in sediments from a shallow lake in Denmark. They found that nitrification began soon after oxygen was introduced into previously anoxic zones indicating that nitrifying organisms can survive periods of anaerobiosis also.

Although it is anticipated that autotrophs are out-competed by heterotrophs for limiting nitrogen supplies, autotrophic nitrification in soils rich in organic matter can occur i.e. autotrophic bacteria are not necessarily washed out by the heterotrophic organisms. This is due to the fact that below a critical C/N ratio, heterotrophic growth is substrate limited and they do not assimilate all the available nitrogen. The surplus nitrogen is then used for autotrophic growth (Verhagen and Laanbroek, 1991).

Moisture content of the soil is also known to be rate limiting for nitrification in many eco – systems. Hastings *et al.*, (2000) found that both heterotrophic and nitrifier counts decreased in acid forest soil during 10 weeks of drought. Once the soil was re-wetted, the nitrifier counts in the upper 6 cm of the soil actually increased beyond their original level.

In many artificial eco-systems such as biofilters and waste treatment plants autotrophic nitrifiers become swamped by the heterotrophic bacteria. Nitrifiers often attach to a surface in their environment, which affects their growth and activity and appears to protect them against inhibitors. Immobilisation e.g. biofilms is a technique that is exploited in practical nitrification applications as it is a good method for retaining such slow growing organisms. Batchelor *et al.*, (1997) examined the difference between the lag times prior to nitrite production in cell suspensions of ammonia oxidisers after starvation to the lag times of cells immobilised on sand or soil particles in continuous flow fixed film reactors. The cell suspensions were harvested during late exponential phase and resuspended in ammonium – free medium for 43 days. It was found that the lag phase prior to nitrite production increased from 8.72 h (no starvation) to 153 h after starvation. The cells in the biofilm were starved by passing the same ammonium – free medium through the reactor and it was found that after 43 days there was no lag phase prior to nitrite

production. It must be noted that although there was no lag phase in the production of nitrites, the time it took to reach pre-starvation steady state values increased as the length of starvation time increased. Also there was a decrease in cell numbers from 7.56×10^7 cells ml^{-1} to 2.43×10^7cells ml^{-1} in the cell suspension during starvation and there was no loss in the number of ammonia oxidisers in the biofilm for the same period of starvation. Both of these results indicate that ammonia-oxidisers are better able to survive unfavourable conditions if they are attached in their environment as biofilms. The reason is thought to be due to the fact that biofilm organisms produce large amounts of extracellular polymeric substances (E.P.S.), which may adsorb ammonium and then gradually release it within the biofilm, maintaining cells at low levels of activity. It could also be related to high cell density – dependent phenomena. In some areas of ecology high cell densities, which can only occur in biofilms, have the ability to respond immediately to changes in nutrient concentrations as organisms with long lag phases would be out – competed e.g. cell suspensions of ammonia – oxidisers (Batchelor et al., 1997).

1.5.5 Methods for assessing nitrifier populations

Numerous methods for the enumeration of nitrifier populations have been developed, however the most probable number method is the most frequently used.

Most probable number method (MPN)

The most probable number method is the easiest method for enumerating nitrifiers. It is based on a statistical method where successive dilutions of the sample are prepared to an extinction dilution where no growth occurs. Three to ten replicates of each dilution are used to estimate the most probable number based on positive and negative growth tubes. The method is based on the assumption that one or more organisms within an inoculant volume are capable of producing a positive result (Underhill, 1990).

Molecular techniques

The polymerase chain reaction amplifies specific sequences of DNA in order to detect microorganisms. It is a very specific and sensitive method and is useful when the organisms are dangerous or difficult to grow in vitro. A drawback associated with this method is the difficulty in purifying nucleic acids from soil environments due to the presence of humic acids and phenolic compounds.

The *amo*A gene, which is found in all ammonia-oxidisers, is generally the sequence of DNA amplified in order to track the ammonia-oxidising community. Malhautier *et al.* (1998) used molecular techniques to assess the microbial population of both a granular activated carbon and a peat biofilter used for the treatment of ammonia. Both filters were inoculated with activated sludge. Over a 102 day experiment when 70 – 80 mg m^{-3} ammonia was supplied to the filter at a flowrate of 0.982 m^3 h^{-1} it was found that the overall diversity of the heterotrophic population decreased by 38% by tracking the 16S rRNA genes. All ammonia oxidising bacteria have an amoA gene that was used to track the nitrifiers and it was found that there was no decrease in the overall diversity of the amoA gene. Nevertheless, there was a shift in groups from groups dominated by Nitrosomonas and Nitrosospira – like amoA genes to groups containing just Nitrosospira – like amoA genes.

16S rRNA – targeted DNA probes, that have been developed for environmental and determinative research have shown that *Nitrosomonas* and *Nitrobacter* cells in activated sludge often cluster together and are found in close proximity of each other, (Verstraete and Phillips, 1998).

Fluorescent antibody technique (FA)

This method involves the preparation of fluorescent antibodies for all known strains of nitrifiers that may be expected in the sample. It has the potential for direct enumeration of nitrifying bacteria but serological diversity is large. A pure culture of each of the strains would have to be obtained in order to develop fluorescent antibodies (Underhill, 1990). According to Malhautier *et al.* (1998) immunofluorescence is more likely to be used for determining serological diversities than for enumerating bacteria.

Dilution plate method

A number of disadvantages are associated with plating nitrifiers due to their slow growth rate. It takes approximately 3 – 4 weeks to get colonies that can only be examined microscopically. Also they are prone to contamination by faster growing heterotrophs. It is far more tedious than the MPN method but the cells are spatially separated at lower dilutions and it is therefore easier to grow several genera of the bacteria (Underhill, 1990). Verhagen and Laanbroek (1991) used both an MPN and FA techniques to enumerate nitrifiers from a continuously fed chemostat. In most cases the FA method yielded higher results than the MPN method. Only 8 – 24 % of the microbes detected using the FA technique were detected using the MPN method. The plate count method for determining heterotrophic populations also yielded lower results than those determined using the FA method.

Alternative methods that monitor nitrifying activity

Jensen *et al.* (1993) used a shielded microsensor for nitrate in freshwater sediments to assess the nitrifying activity in the soil. They found that the microsensor gave quick rapid results on how the nitrifying community responded to changes in the environmental conditions.

Perfusion technique

Often, the nitrifying activity or the overall nitrifying potential of soil is measured instead of direct enumerations of the nitrifiers. Traditionally this was achieved by the soil incubation technique i.e. soil supplemented with ammonium was incubated at the required temperature and moisture content and samples of the soil were taken at regular intervals and monitored for ammonium, nitrite, nitrate and sometimes microbial concentrations. The perfusion technique involves circulating a solution containing a known concentration of ammonium through a soil column and monitoring the effluent for ammonium, nitrite and nitrate over time. The perfusion technique minimises the losses in soil volumes that inevitably occurs in the incubation technique, (Prosser, 1986).

1.5.6 Recent technologies involving nitrification – denitrification

ANAMMOX

This process involves the conversion of ammonium to di-nitrogen gas under anaerobic conditions. It involves a pre-nitrification step that is blocked at nitrite and then the nitrite is used as the electron carrier. ANAMMOX is an autotrophic process and because only part of the ammonium needs to be nitrified, (to yield nitrite for sufficient electron acceptors), this method reduces both the oxygen and carbon requirements of traditional wastewater treatment plants. The organism responsible for the direct conversion of ammonium to di-nitrogen gas is not fully characterised (Verstaete and Phillips, 1998).

***SHARON* (Single reactor High Ammonia Removal Over Nitrite)**

Some of the ammonium in nutrient - rich wastewater is converted to nitrite and then the mixture of ammonium and nitrite is passed on to the ANAMMOX process for conversion to di-nitrogen gas. Generally it is difficult to block nitrification once nitrite is produced but SHARON exploits the fact that *Nitrobacter* has a lower growth rate than *Nitrosomonas* at high temperatures (Verstraete and Phillips, 1998).

***De-ammonification* (aerobic de-nitrification)**

This is the conversion of ammonium to di-nitrogen gas without the stoichiometric requirement for an electron donor under limited oxygen conditions. Verstaete and Phillips (1998) reported a maximum 58% oxidation of ammonium at 0.3 kPa dissolved oxygen. A disadvantage associated with this process is that under limited oxygen conditions nitric and nitrous oxide are produced by ammonia-oxidisers.

Waste gas treatment technologies

Bioscrubbers, trickling filters and biofilters exploit the ability of nitrifying bacteria to convert ammonia to nitrate, which can be further denitrified under anaerobic conditions to yield non-toxic products that can be released into the atmosphere.

1.6 Objectives of this study

Ammonia was chosen as a model substrate as it is a known constituent of odorous gases from intensive farming practices. It is also emitted from numerous other

industrial sources and is widely recognised as an acidifying pollutant. National and international regulations have been implemented in recent years to minimise the release of gaseous pollutants into the environment. To this end a laboratory-scale biofilter was designed and operated for the purpose of removing ammonia from a gas stream.

Aims

- To design and operate a laboratory-scale biofilter
- To study the removal of gaseous ammonia using a peat biofilter.
- To look at the effects of shock-loading and shut-down periods on removal efficiency
- To monitor heterotrophic and autotrophic microbial populations throughout the biofiltration experiment
- To look at the effects of supplying high concentrations of ammonia on the packing material

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 The biofilter structure

The biofilter used in this study was constructed from perspex with dimensions 0.6 m in height and 0.2 m in inner diameter (Fig. 7). Acrylonitrile butadiene styrene was used for both the lid and the base, the lid of which was detachable. A stainless steel sieve plate was placed 0.05 m from the base of the system, which supported the packing material and ensured even distribution of the inlet air stream. Two sample ports, 0.025 m inner diameter, which were located at 0.2 m and 0.4 m of the filters' height, were used to obtain packing material samples. They were fixed in place with Nova – Seal Silicone Gel and during the operation of the filter the sample ports were sealed with rubber bungs. Excess water that percolated from the system was removed via a collection duct and a Swagelock stainless steel valve at the base of the biofilter. The biofilter was packed to a height of 0.45 m with 8 kg of packing material. The final packed volume was 0.014 m^{-3}.

2.1.2 Packing material

Peat, supplied by Bord na Mona, Newbridge, Co. Kildare, Ire. was used as the packing material in the biofilter. The peat was in pellet form. The individual pellets had a diameter ranging from less than 4 mm to 40 mm in diameter. The peat had a pH of pH 5.85 and had a moisture content of 20.7 ± 1.1 %.

2.1.3 Source of nitrifying bacteria

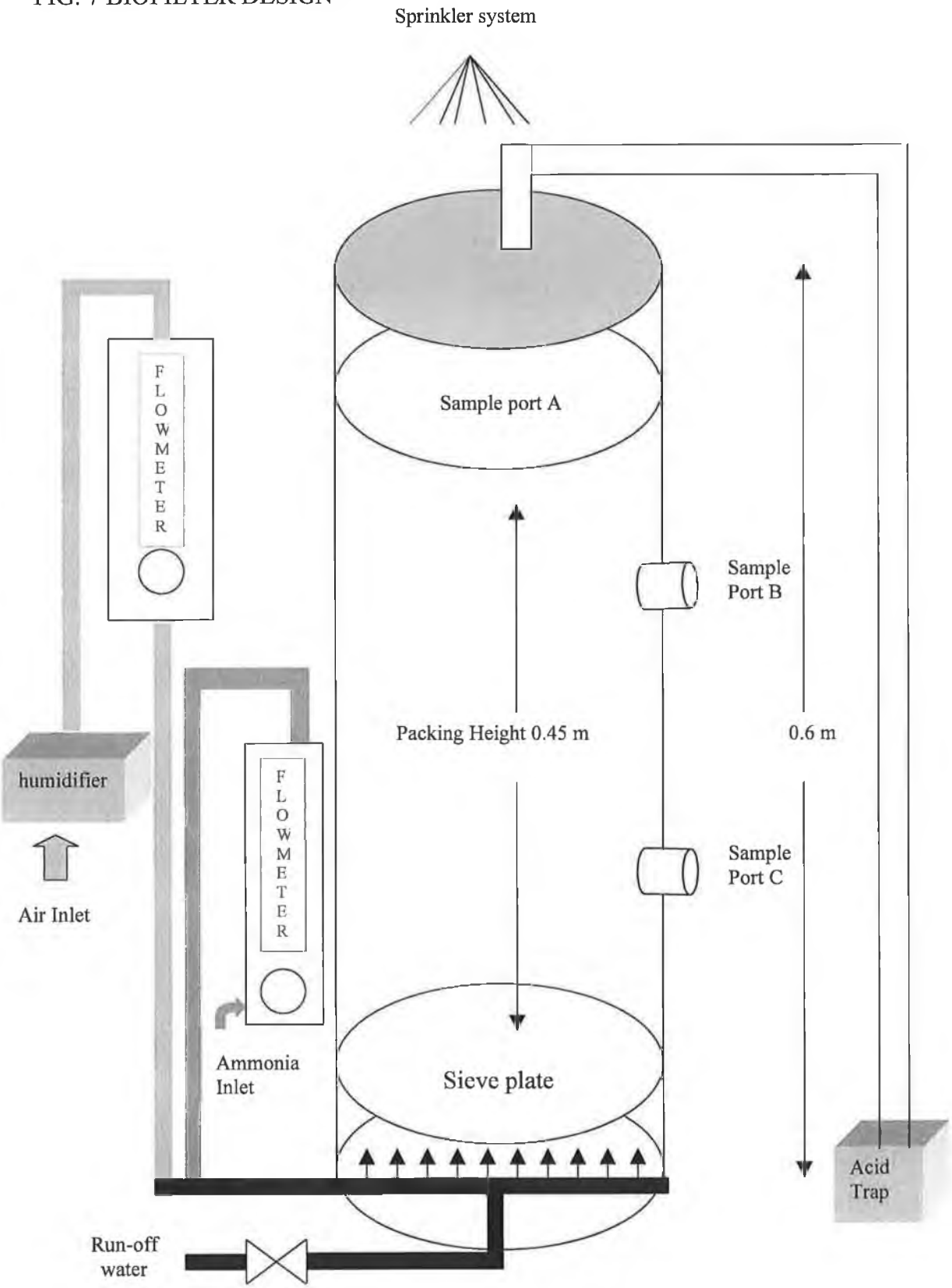
(a) Nitrifying activated sludge was obtained from the waste treatment plant of a local pharmaceutical company.

(b) Pure cultures of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* were obtained from The National Collection of Industrial and Marine Bacteria (NCIMB Ltd.), Aberdeen, Scotland.

2.1.4 Gaseous ammonia

BOC gases, Dublin, supplied anhydrous ammonia of 99.98 % purity.

FIG. 7 BIOFILTER DESIGN



2.1.5 Microbiological growth media

All media used for enumerating microorganisms were obtained from Oxoid and were sterilised at 121^{0}C for 15 minutes, unless otherwise stated.

Plate count / cycloheximide agar

The plate count agar was prepared according to the manufacturers' instructions. Following sterilisation, the medium was allowed to cool. Cycloheximide, dissolved in 100% ethanol to a concentration of 10 mg ml^{-1}, was sterilised using Gelman Laboratory Acrodiscs®, 0.2 µm syringe filters and added to the agar immediately prior to pouring. The final concentration of cycloheximide in the medium was 0.15 mg ml^{-1}.

Malt extract / chloramphenicol agar

The malt extract agar was prepared according to the manufacturers' instructions and sterilised by autoclaving at 115^{0}C for 10 minutes. Following sterilisation the medium was allowed to cool. Chloramphenicol, dissolved in methanol to a concentration of 10 mg ml^{-1}, was sterilised using Gelman Laboratory Acrodiscs®, 0.2 µm syringe filters and added to the agar immediately prior to pouring. The final concentration of chloramphenicol in the agar was 0.1 mg ml^{-1}.

Ammonium-calcium-carbonate medium

Ammonium-calcium-carbonate medium was prepared as outlined by Alexander and Clark (1965). The reagents as described below were combined in distilled water.

Reagent	**g L^{-1}**
$(NH_4)_2SO_4$	0.5
K_2HPO_4	1.0
$FeSO_4.7H_2O$	0.03
NaCl	0.3
$MgSO_4.7H_2O$	0.3
$CaCO_3$	7.5

Media used for the cultivation of the nitrifying bacteria

Ammonia-oxidising bacteria

The medium for the growth of ammonia-oxidising bacteria was prepared as described in The Catalogue of Strains by NCIMB Ltd. (Young and M^{c}Farlane, 1994).

The following ingredients were dissolved in distilled water.

Reagent	mg L^{-1}
$(NH_4)_2SO_4$	235
KH_2PO_4	200
$CaCl_2.2H_2O$	40
$MgSO_4.7H_2O$	40

A stock solution containing 0.5 mg ml^{-1} $FeSO_4.7H_2O$, 0.5 mg ml^{-1} NaEDTA and 0.5 mg ml^{-1} phenol red was prepared in distilled water. 1 ml of solution was added to the medium prior to autoclaving.

Nitrite-oxidising bacteria

Medium for the growth of nitrite-oxidising bacteria was prepared by dissolving the ingredients below in distilled water as outlined in The Catalogue of Strains by NCIMB Ltd. (Young and M^{c}Farlane, 1994).

Reagent	mg L^{-1}
$NaNO_2$	247
KH_2PO_4	200
$CaCl_2.2H_2O$	40
$MgSO_4.7H_2O$	40

A stock solution containing 0.5 mg ml^{-1} $FeSO_4.7H_2O$, 0.5 mg ml^{-1} NaEDTA and 0.5 mg ml^{-1} phenol red was prepared in distilled water. 1 ml of solution was added to the medium prior to autoclaving.

2.1.6 Source of chemicals

Chemicals were obtained from a number of sources including Reidel-de-Haen, BDH, Sigma and Aldrich.

2.2 Methods

2.2.1 Treatment of the packing material

2.2.1.1 Sieving

Prior to packing, the peat was sieved with a sieve shaker (Pascall Engineering Co. Ltd., Sussex, England), to remove fines and particles less than 4 mm in diameter.

2.2.1.2 Neutralisation and moisture control

The peat was soaked in tap water for 48 hours to increase the moisture content. In order to neutralise the pH of the peat, 0.9 g $Ca(OH_2)$ Kg^{-1} peat was added to the soaking water, which is a modified version of that described by Hartikainen *et al.* (1996).

2.2.1.3 Inoculation

The peat was inoculated with activated sludge at the beginning of the experiment when 200 ml of the inoculum was percolated through the peat bed. The biofilter was re-inoculated towards the end of the experiment with 750 ml activated sludge, which was also percolated through the bed.

2.2.2 Growth of the nitrifying bacteria

Ammonia –oxidising bacteria

The ammonia oxidising bacteria were cultivated as described in The Catalogue of Strains by NCIMB Ltd. (Young and M°Farlane, 1994). Sterile 5 % Na_2CO_3 was added to the medium until it turned a pale pink. Further 5 % Na_2CO_3 was added during incubation to restore the pink colour. Growth was complete when no further colour changes occurred. The cells were incubated in the dark at 28^0C on a Denley rotary shaker table at 110 rpm.

Nitrite oxidising bacteria

The nitrite oxidising bacteria were cultivated as described in The Catalogue of Strains by NCIMB Ltd. (Young and M^{c}Farlane, 1994). The cells were incubated at 28^{0}C on a Denley rotary shaker table at 110 rpm. Growth was monitored by the removal of nitrite from the medium using Greiss-Ilosvay's reagent (BDH, Ire.).

2.2.3 Biofilter operation

The biofilter was operated discontinuously in an upflow mode.

2.2.3.1 Gas supply to the biofilter

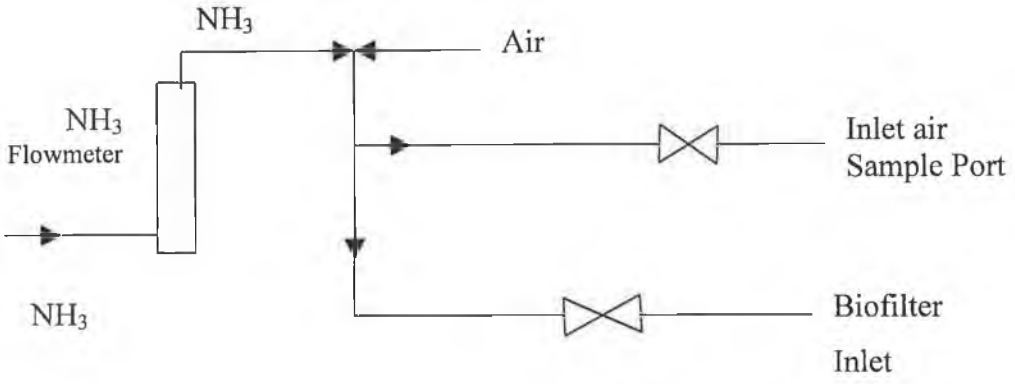
Ammonia supply to the biofilter

Ammonia was used as the substrate for biofiltration. Stainless steel tubing was required to transport the ammonia from the cylinder to the biofilter. Ammonia supply to the biofilter was regulated using a BOC stainless steel, single stage cylinder regulator, HP 1500 Series with a body purge and the flowrate was controlled with a Manotherm flowmeter (5 cm^3 min^{-1} to 100 cm^3 min^{-1}) as illustrated in Fig.8. Flow of ammonia to the biofilter varied from 5 cm^3 min^{-1} to 15 cm^3 min^{-1} in order to vary the inlet ammonia concentration. The ammonia flowrate used for each run was determined empirically.

Air supply to the biofilter

Benchtop compressed air was used to dilute the gaseous ammonia. The flowrate of the compressed air was controlled using a BOC flowmeter (0 – 54 L min^{-1}). Throughout the research the air flowrate was maintained at 18 - 23 L min^{-1}.

Figure 8 Ammonia Inlet



2.2.3.2 Moisture Control

Moisture control was achieved within the biofilter by pre-humidifying the inlet gas and by periodically sprinkling tap water directly on to the surface of the peat bed. Humidification of the inlet gas was achieved by bubbling the compressed air through water before it was used to dilute the ammonia and supplied to the biofilter.

Relative humidity measurement

The relative humidities of the gas supplied and the gas that emerged from the biofilter were read from a psychrometric chart (Fig. 9), based on the wet and dry bulb temperatures. Both wet bulb and dry bulb temperatures of each gas stream were measured using a Checktemp temperature probe (AGB, Dublin, Ire.).

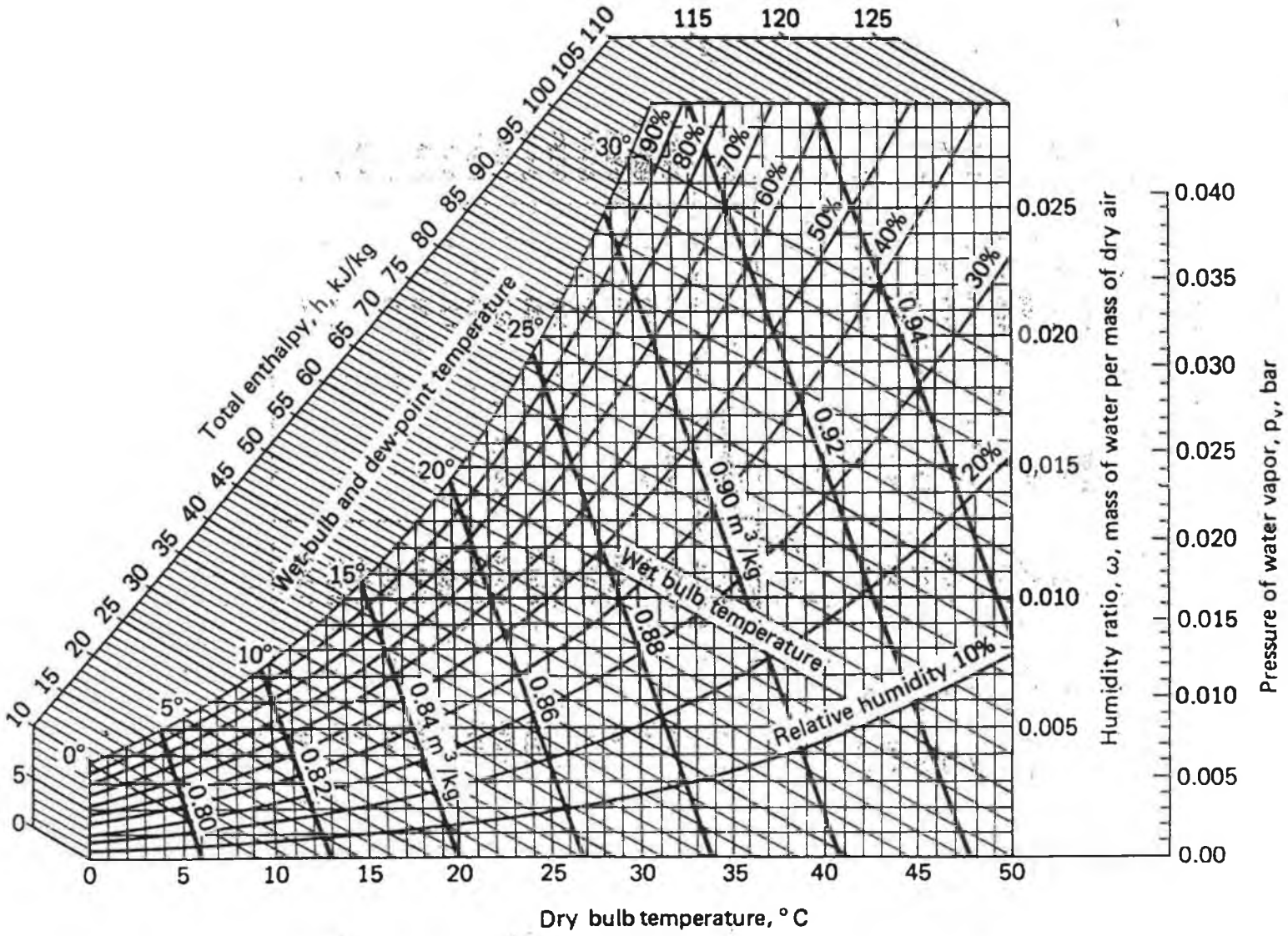
Moisture measurement

(a) The moisture of the peat was determined by wet weight analysis as described by Yani *et al.,* (1998). 10 g of peat was oven dried at 105 ^{0}C for 24 h. The difference between the weight before and the weight after oven drying was used to calculate the amount of moisture lost and equated to the original level of moisture per sample.

% Moisture = weight before drying – weight after drying / weight before dryingx100

(b) Water was added to the biofilter bed based on visual inspection.

Fig. 9 Psychrometric chart (Wark, 1977)



2.2.3.3 Ammonia Measurement

2.2.3.3.1 Ammonia collection

Gaseous ammonia was trapped in 0.05M H_2SO_4. Four gas-washing Dreschel bottles placed in series, containing 50 ml acid each, were required to ensure complete removal of ammonia from the gas stream. The method of trapping ammonia was a modification of the method documented by Harrison, (1986). Three minutes sampling time was found to be optimal.

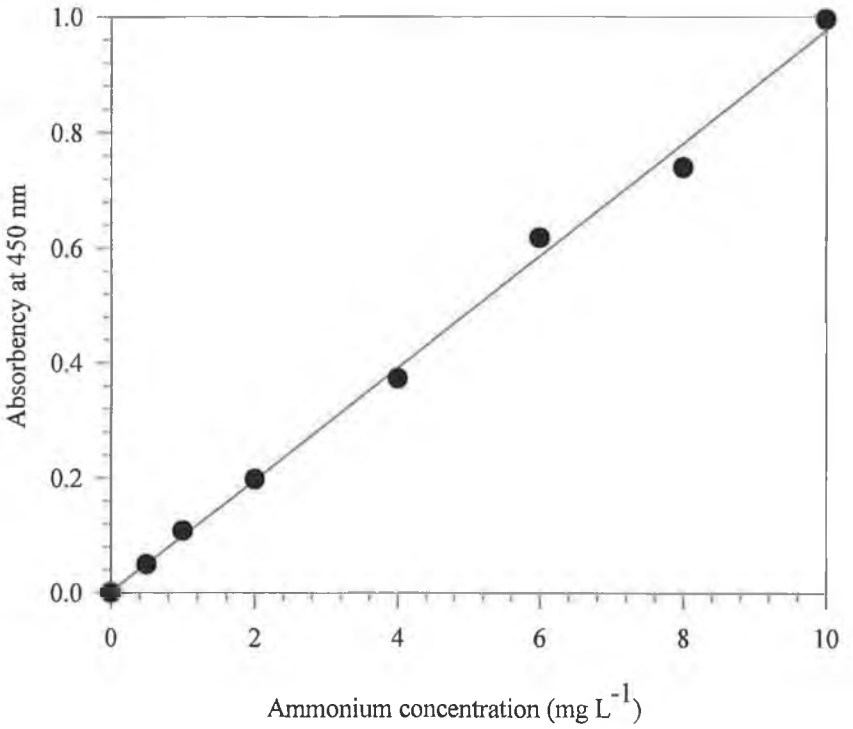
Trapping of Gaseous Ammonia

$$2\ NH_3\uparrow + 2\ H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2SO_4$$

2.2.3.3.2 Nessler Method for ammonia concentration determination

Ammonia concentration was determined by the Nessler method, as described in Standard Methods for the Examination of Water and Wastewater (Greenberg *et al.*, 1992). A standard curve was constructed within the linear range of 0 – 10 mg L^{-1} ammonium using NH_4Cl solution (Fig. 10). Both standards and samples were treated as undistilled samples. 1 ml $ZnSO_4$ was added to 100 ml sample and the pH of the solution was increased to pH 10.5 by the addition of 0.5 ml 6 N NaOH. A heavy precipitate formed, which was removed by filtering through Whatmann No. 2 filter paper. A drop of EDTA reagent followed by 2.0 ml Nessler reagent was added 50 ml of the filtrate. Colour was allowed to develop for 10 min and absorbency was measured at 450 nm.

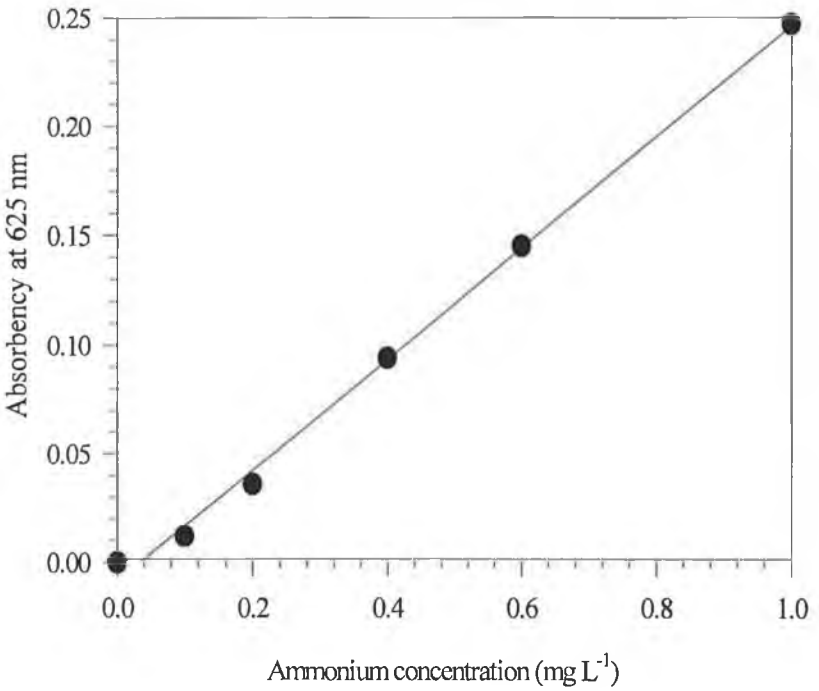
Fig. 10 Ammonia standard curve using Nessler method



2.2.3.3.3 Indophenol Method for ammonia concentration determination

The catalysed indophenol-blue method as outlined in Handbook of Air Pollution Analysis (Harrison, 1986) was used to determine ammonia concentrations. A standard curve was constructed within the linear range of 0 – 1 mg L^{-1} ammonium using $(NH_4)_2SO_4$ solution (Fig. 11). 10 ml phenol nitroprusside and 10 ml alkaline hypochlorite were added to 5 ml sample. Phenol nitroprusside was prepared by mixing 5 g of phenol and 20 mg sodium nitroprusside in 500 ml de-ionised water and the alkaline hypochlorite was prepared by adding 4.2 ml sodium hyopchlorite to 10 g L^{-1} NaOH. Both samples and standards were treated in the same manner. The colour of the samples and standards were allowed to develop for 30 min and absorbency was measured at 625 nm.

Fig. 11 Ammonia standard curve using indophenol method



2.2.3.3.4 Ion Specific Electrode Method for ammonia concentration determination

An Orion ammonia specific electrode model 9512 (Orion Research Incorporated, Boston, MA, USA) was used to determine ammonia concentrations. The electrode

was calibrated with reference to NH_4Cl standards. The standards used to quantify the gaseous ammonia were prepared in the absorbing solution. All other standards were prepared in distilled water. Standards used were in the range 0 – 1000 mg NH_3 L^{-1}. Samples were diluted with 2% v/v ionic strength adjuster (5M NaOH). Ammonia concentration in the sample was determined using the direct readout capability of an Orion benchtop pH/ISE meter, (model 920A).

2.2.3.3.5 Determination of ammonia concentration

Sample Calculation to determine gaseous ammonia concentration

Ammonia concentration in the trapping acid was expressed in terms of mg NH_3 L^{-1} acid. In order to determine the concentration of gaseous ammonia in the air streams, the value was converted to mg NH_3 m^{-3} air.

1. The amount of ammonia in each of the gas-washing Dreschel bottles in terms of mg ammonia was calculated by multiplying the ammonia concentration (mg ammonia L^{-1} acid) in each flask by the volume of trapping acid in that flask i.e. 50 ml of 0.05 M H_2SO_4.

   - *Total mg ammonia in each flask*

     = Concentration (mg NH_3 L^{-1} acid) x volume trapping acid (L acid)

2. The total amount of ammonia trapped by the acid (sum of amounts of ammonia in each flask) was divided by the total volume of air sampled to yield ammonia concentrations in terms of mg ammonia m^{-3} gas.

   - *Volume of air sampled = Flowrate (L min^{-1}) x Sampling time (min.)*
   - ***Concentration of ammonia (mg m^{-3})***

     = Total amount of ammonia / vol. air sampled

- Ammonia concentration in the percolate

Percolate samples were treated as previously described for ammonia concentration determination. The concentration was expressed as mg ammonia L^{-1} percolate.

- Ammonia adsorbed by the peat

Samples (10g) of peat were taken from sample ports A, B and C. In order to de-sorb the ammonium from the peat, the samples were placed in 50 ml 0.1M H_2SO_4 and shaken for 48 hours at 150 rpm on a Denley orbital shaker at room temperature. The concentration of ammonia in solution was determined as previously described. From the concentration of ammonia in solution the amount of ammonia present was calculated and the value was reduced to the mass of the peat sample. It was expressed as mg ammonia g^{-1} peat.

2.2.3.4 Nitrite and nitrate determination

The levels of nitrite and nitrate were measured both in the percolate and on the peat. Nitrate on the peat was measured by taking 10g samples of peat from sample ports A, B and C. The samples were placed in 30 ml 0.01 M $CaSO_4$ solution and shaken at 150 rpm on a Denley orbital shaker at room temperature in order to extract the nitrate from the samples. After 15 min the samples were filtered through Whatmann No. 2 filter paper and the nitrate concentration in the filtrate was determined (http://bluehen.ags.udel.edu/deces/prod_agric/chap4-95.htm 05-01-2001). From the concentration of nitrate in the filtrate the amount of ammonia present was calculated and the value was reduced to the mass of the peat sample. It was expressed as mg nitrate g^{-1} peat. Nitrite and nitrate were measured using either of the following methods.

2.2.3.4.1 Diphenylamine method

A diphenylamine solution was prepared by dissolving 0.2 % (w/v) diphenylamine powder in concentrated sulphuric acid. 30 % v/v of the diphenylamine solution (indicator) was added to the sample. In the presence of nitrites / nitrates the indicator turned blue (Morgan, 1930).

2.2.3.4.2 Ion specific electrode method

Nitrate concentration was measured using an Orion ion selective electrode connected to a benchtop pH/ISE meter (model 920A). The electrode was calibrated with reference to KNO_3 standards with concentrations of 100 and 1000 mg NO_3^- L^{-1} H_2O.

Samples and standards were diluted with 2% ionic strength adjuster (2M $(NH_4)_2SO_4$). Concentrations of nitrate were determined using the direct readout capability of the pH/ISE meter.

2.2.3.5 pH determination

pH was measured using an Orion Triode™ pH electrode model 91–57 BN connected to an Orion benchtop pH meter model 420A. The pH of the percolate was measured directly by immersion of the probe in the run-off liquid. The pH of the packing material was determined by a modified version described by Martin *et. al*, (1996). The value of pH was measured in distilled water that contained 1 part peat per 5 parts water, which was mixed for 15 min on a Denley Orbital Shaker table at room temperature.

2.2.4 Microbial analyses

2.2.4.1 Treatment of peat samples

Samples of the peat (10g) were placed in 90 ml of sterile quarter-strength Ringers' (Oxoid) solution. The samples were shaken at 150 rpm for 10 min. at room temperature on a Denley orbital shaker table. Suitable dilutions were prepared for enumeration of bacterial and fungal cells by the pour plate method. The nitrifying bacteria on the samples were also enumerated using a most probable number method.

2.2.3.2 The plate count method

Suitable dilutions of the sample were tested for the presence of bacteria and fungi using the pour plate method. 1ml aliquots were plated in triplicate using the appropriate agar and incubation conditions. The bacteria were enumerated using plate count / cycloheximide agar; they were incubated for 48 h at 30 ^{0}C. Fungal cells were enumerated on malt extract / chloramphenical agar; they were incubated at 25 ^{0}C for 72 h. The results were expressed as colony forming units (cfu) per gram peat.

2.2.3.3 Most probable number (MPN) method

Two versions of the MPN method were used – the macro-method in test tubes and the micro-method in micro-titre plates. Pure cultures of *Nitrosomonas europaea* and

Nitrobacter winogradskyi were used as positive controls in the MPN method. Sterile quarter-strength Ringers' solution was used as a negative control.

Macro-method

10-fold dilutions of each sample were prepared in sterile quarter-strength Ringers' solution. 1 ml of each sample dilution was added to a test tube containing 3 ml ammonium-calcium-carbonate media. Five replicas of each dilution were prepared. The tubes were incubated at 28^0C for three weeks. Growth was determined by the production of nitrites and/or nitrates, which were detected using the diphenylamine method (Section 2.2.3.4.1). The most probable number of organisms was calculated from a table as described in Cochran (1950) (Table 8).

Microtechnique

The samples were prepared as described for the macro method. 0.05 ml of ammonium-calcium-carbonate medium was placed into 5 x 5 wells of a sterile microtitre plate (Costar 96-well cell culture plates). 0.05 ml of sample for the appropriate dilution was plated to give five replicas of each sample. The microtitre plates were incubated at 28^0C for three weeks (Rowe, *et al.,* 1977). Growth was determined by the production of nitrites or nitrates, which were detected using the diphenylamine method (Section 2.2.3.4.1). The most probable number of organisms was calculated from a table as described in Cochran (1950) (Table 8).

Table 8 Table of most probable numbers for use with 10-fold dilutions and 5 tubes per dilution (Cochran, 1950).

		Most probable number for indicated values of P_3					
P_1	P_2	0	1	2	3	4	5
0	0	-	0.018	0.036	0.054	0.072	0.090
0	1	0.018	0.036	0.055	0.073	0.091	0.110
0	2	0.037	0.055	0.074	0.092	0.110	0.130
0	3	0.056	0.074	0.093	0.110	0.130	0.150
0	4	0.075	0.094	0.110	0.130	0.150	0.170
1	5	0.094	0.110	0.130	0.150	0.170	0.190
1	0	0.020	0.040	0.060	0.080	0.100	0.120
1	1	0.040	0.061	0.081	0.100	0.120	0.140
1	2	0.061	0.082	0.100	0.120	0.150	0.170
1	3	0.083	0.100	0.130	0.150	0.170	0.190
1	4	0.110	0.130	0.150	0.170	0.190	0.220
1	5	0.130	0.150	0.170	0.190	0.220	0.240
2	0	0.045	0.068	0.091	0.120	0.140	0.160
2	1	0.068	0.092	0.120	0.140	0.170	0.190
2	2	0.093	0.120	0.140	0.170	0.190	0.220
2	3	0.120	0.140	0.170	0.200	0.220	0.250
2	4	0.150	0.170	0.200	0.230	0.250	0.280
2	5	0.170	0.200	0.230	0.260	0.290	0.320
3	0	0.078	0.110	0.130	0.160	0.200	0.230
3	1	0.110	0.140	0.170	0.200	0.230	0.270
3	2	0.140	0.170	0.200	0.240	0.270	0.310
3	3	0.170	0.210	0.240	0.280	0.310	0.350
3	4	0.210	0.240	0.280	0.320	0.360	0.400
3	5	0.250	0.290	0.320	0.370	0.410	0.450
4	0	0.130	0.170	0.210	0.250	0.300	0.360
4	1	0.170	0.210	0.260	0.310	0.360	0.420
4	2	0.220	0.260	0.320	0.380	0.440	0.500
4	3	0.270	0.330	0.390	0.450	0.520	0.590
4	4	0.340	0.400	0.470	0.540	0.620	0.690
4	5	0.410	0.480	0.560	0.640	0.720	0.810
5	0	0.230	0.310	0.430	0.580	0.760	0.950
5	1	0.330	0.460	0.640	0.840	1.100	1.300
5	2	0.490	0.700	0.950	1.200	1.500	1.800
5	3	0.790	1.100	1.400	1.800	2.100	2.500
5	4	1.300	1.700	2.200	2.800	3.500	4.300
5	5	2.400	3.500	5.400	9.200	16.000	-

2.3 Physical parameters

- Packed volume

The packed volume is the volume of packing material in the biofilter.

$$\mathbf{V\ (m^3) = \pi\ r^2\ h}$$

E.g. radius = 0.1 m

Packed height = 0.45 m

Volume (m^3) = $\pi\ (0.1)^2\ (0.45)$

= 0.014 m^3

- Packed density

The packed density is the mass of the packing material per unit volume of the packing material.

Density (Kg m^{-3}) = mass / bed volume

E.g. packing mass = 8 Kg

packed volume = 0.014 m^3

D (Kg m^{-3}) = 8 Kg / 0.014 m^3

= 571.4 Kg m^{-3}

- Empty bed contact time

The empty bed contact time is the residence time of the gas in the biofilter (assuming no resistance to flow).

EBCT (s) = packed volume / flowrate

E.g. packed volume = 0.014 m^3

Flowrate = 18 L min^{-1} = 0.0003 $m^3\ s^{-1}$

EBCT (s) = 0.014 m^3 / 0.0003 $m^3\ s^{-1}$

= 47 s

- Amount of ammonia supplied in each run

  The amount of ammonia supplied in each run was calculated by multiplying the inlet concentration by the total volume of gas supplied.

Inlet ammonia concentration (mg m^{-3}) x total vol. gas in that run (m^{-3})

E.g.	total vol. gas	= Flowrate x running time
	Flowrate	= 18 L min^{-1}
	running time	= 175 min^{1}
	total vol. gas	= 3150 L = 3.15 m^{-3}
	inlet conc.	= 1833.5 mg m^{-3}
⇒	amount ammonia	= 1833.5 mg m^{-3} x 3.15 m^{-3}
		= 5775.5 mg

- Mass loading

  The mass load can be described as the chemical mass load supplied to the biofilter per unit packing mass.

Mass Loading (g NH_3 Kg^{-1} peat h^{-1})

= Flowrate x inlet conc. / Mass of packing material

E.g.	Flowrate	= 18 LPM = 1080 L h^{-1} = 1.08 m^{-3} h^{-1}
	Inlet concentration	= 1833.5 mg m^{-3}
	Mass packing mat.	= 8 Kg

Note: Although the mass of the peat bed varied as a result of the removal of peat samples the mass was assumed to be 8 Kg for calculation purposes.

Mass load	= 1.08 m^{-3} h^{-1} x 1833.5 mg m^{-3} / 8 Kg
	= 247.5 mg NH_3 Kg^{-1} peat h^{-1}
	= 0.248 g NH_3 Kg^{-1} peat h^{-1}

- Elimination capacity

The elimination capacity can be described as chemical mass removal rate per unit bed mass.

Elimination Capacity (g NH_3 Kg^{-1} dry peat h^{-1})

= Flowrate (m^3 h^{-1}) x (inlet conc. – outlet conc.) / Mass of packing

E.g. Flowrate = 18 LPM = 1.08 m^3 h^{-1}

Inlet concentration = 1833.5 mg m^{-3}

Outlet concentration = 127 mg m^{-3}

Mass Packing Mat. = 8 Kg

Elimination capacity = 1.08 (m^3 h^{-1})x(1833.5 (mg m^{-3})–127 (mg m^{-3})) / 8 Kg

= 384 mg NH_3 Kg^{-1} peat h^{-1}

= 0.384 g NH_3 Kg^{-1} peat h^{-1}

- Removal efficiency

The removal efficiency is a measurement of the performance of the biofilter.

Removal Efficiency (%) = ((Inlet conc. – outlet conc.) / inlet conc.) x 100

E.g. Inlet concentration = 1833.5 mg m^{-3}

Outlet concentration = 127 mg m^{-3}

Removal efficiency = 93.1 %

2.4 Data analysis

All analyses were performed in triplicate. The standard error mean was calculated as

$$S_m = \sigma / \sqrt{n}$$

where S_m is the standard error mean, σ is the standard deviation and n is the sample size by the computer software package Microsoft Excel 2000 (Microsoft Corporation).

Regression analysis was used to determine the line of best fit. The degree of correlation of the data was determined by the correlation co-efficient, r^2 , which was calculated by the computer software package Sigma Plot (version 5.0), (Jandel Corporation).

3.0 RESULTS

3.1 Biofilter operation

The removal of ammonia by a biofilter was studied for a period of 297 days. The main operating parameters including gas supply and the control of temperature and humidity in the system are described below.

3.1.1 Ammonia supply

The biofilter was operated in a discontinuous mode with three distinct stages of operation. Each stage differed by the average inlet concentration of ammonia supplied in each run during that stage (Fig. 12). During the initial stage, which comprised day 1 to day 34, the average inlet ammonia concentration supplied to the biofilter was 13.9 mg m^{-3}, which corresponded to a mass load of 0.0023 g NH_3 Kg^{-1} peat h^{-1}. The average inlet concentration of ammonia supplied to the filter during stage 2 was 564.8 mg m^{-3} and the corresponding mass load was 0.082 g NH_3 Kg^{-1} peat h^{-1}. Stage 2 was conducted between day 51 and day 185. During stage 3, which took place between day 186 and day 297, an average inlet concentration of 2226.0 mg m^{-3} ammonia was supplied to the peat bed. The average inlet concentration corresponded to a mass load of 0.301 g NH_3 Kg^{-1} peat h^{-1}.

Concentrations of ammonia were measured throughout the project using the ammonia ion specific electrode. This method was chosen above the colorimetric methods for sensitivity reasons and was used to monitor ammonia concentrations in the gas supplied and the gas that emerged from the biofilter, together with the ammonia concentrations in the percolate and the amount adsorbed by the peat.

The flowrate of the gas supplied to the biofilter varied from 23 L min^{-1} in stage 1 to 18 L min^{-1} in stage 3 (Table 9). The flowrates used were chosen to ensure a suitable empty bed contact time (EBCT) for the gas. The EBCT values for stages 1 – 3 are outlined in Table 9 and range from 37 s in stage 1 to 47 s in stage 3.

Fig. 12 Ammonia inlet concentrations supplied to the biofilter for three stages of operation

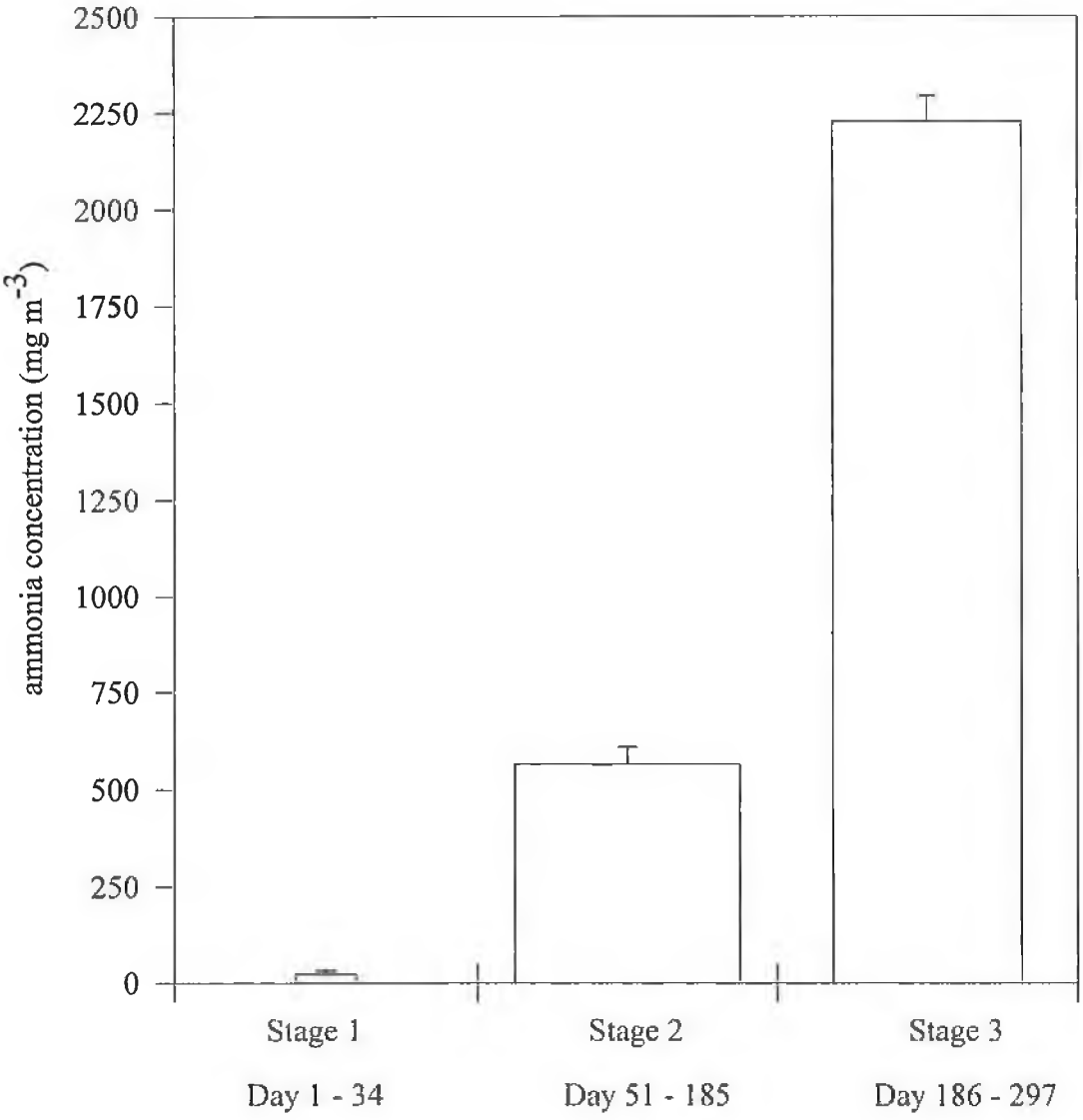


Table 10 The temperature and relative humidity of the inlet and outlet gas

Day	Inlet temp.	Inlet R.H.	Outlet temp.	Outlet R.H.
13	22	65 – 70	23.1	80 – 85
18	19.6	50 – 55	20.0	75 – 80
20	19.9	60 – 65	20.5	70 - 75
29	21.8	55 - 60	22.6	65 – 70
32	18.9	50 – 55	19.6	65 – 70
34	19.7	55 – 60	20.1	85 – 90
51	21.1	60 – 65	22.5	90 – 95
55	24.9	60 – 65	25.1	90 – 95
61	22.6	60 – 65	23.5	80
63	21.4	60	22.2	70 – 75
67	19.4	55 - 60	19.7	90
69	20.5	55 - 60	20.7	85 – 90
70	20.1	60 – 65	20.6	70 – 75
75	21.1	55 – 60	22.1	65 – 70
84	20.3	50 – 60	21.9	65 – 70
92	18.2	55	20.2	60 – 65
94	19.1	55 – 60	19.1	65 – 70
115	19.2	60 – 65	20.7	70
129	18.2	60 – 65	19.4	75 – 80
136	19.2	55 – 60	19.2	75
181	18.7	55	19.4	65
182	19.1	55	19.6	85 – 90
185	19.4	60 – 65	19.4	80 – 85
188	15.5	50 – 55	18.4	80
207	16.3	50	18.0	70 - 75
234	17	45 – 50	19.6	70 – 75
235	16.4	45 - 50	16.8	70 –75
257	18.2	55 – 60	18.7	80 – 85
268	20.2	60 – 65	20.5	70 - 75
276	19.4	60 – 65	20.0	75 – 80
297	19.7	55 - 60	20.4	70 - 75

3.2 Stage 1

The ability of the biofilter to eliminate ammonia from a waste gas stream, under stage 1 conditions, as outlined below, was studied. The microbiological changes within the system together with the removal efficiency were included in the investigation.

3.2.1 Ammonia removal during stage 1

The discontinuous mode by which ammonia was supplied to the biofilter is presented in Figure 13. Stage 1 of the biofilter operation extended from day 1 to day 35. The ammonia was supplied at intervals, as depicted in the bar-chart (Fig. 13). Both the inlet concentration and the period of operation of each run are accounted for by the total amount of ammonia supplied to the peat bed during each run.

The amount of ammonia supplied to the biofilter increased gradually between day 1 and day 20 from 1.7 mg on day 1 to 13.6 mg on day 20 (Fig. 13). During that period the mass loads varied from 0.0005 to 0.0016 g NH_3 Kg^{-1} peat h^{-1}. Untreated ammonia emerged from the peat bed in the form of ammonia in the outlet gas stream. Between 0.53 mg and 4.72 mg ammonia emerged in the outlet air, which corresponded to a removal efficiency below 70 % (Table 11).

A maximum mass load of 0.0077 g NH_3 Kg^{-1} peat h^{-1} was supplied to the filter on day 29, when 61.25 mg of ammonia was supplied during one run. The removal efficiency increased to 97 %, with only 1.85 mg ammonia emerging in the outlet air. This illustrated that following an initial acclimation period, the removal efficiency of the biofilter not only increased but the biofilter was capable of dealing with shock loads. Similar mass loads were supplied for the remainder of Stage 1 and the removal efficiency of the biofilter remained high.

Nitrogen was lost from the system in the percolate in the form of ammonium, which was measured as ammonia, and nitrates.

Ammonia emerged in the percolate throughout stage 1. The percolate that emerged on day 13 and day 20 contained 5.30 mg and 5.46 mg ammonia respectively at concentrations of 378 mg L^{-1} and 546 mg L^{-1} (Table 11).

The concentration of ammonia detected in the percolate reached a peak on day 20 and then decreased. The decrease in ammonia detected in the percolate after day 20

corresponded with the acclimation of the system as indicated by the removal efficiency.

During stage 1 the presence of nitrites and/or nitrates were detected using the diphenylamine indicator. Whenever percolate was collected from the system, nitrites and/or nitrates were always present. The presence of such products suggested the presence of nitrifying microorganisms in the peat bed. The pH of the percolate ranged from pH 6.21 to pH 8.00 during stage 1 (Table 11). The pH rose initially corresponding to the increase in the concentration of ammonia in the percolate at day 20 and then decreased correspondingly, further indicating the presence of nitrification in the system.

Fig. 13 Ammonia supplied to the biofilter during stage 1 of operation

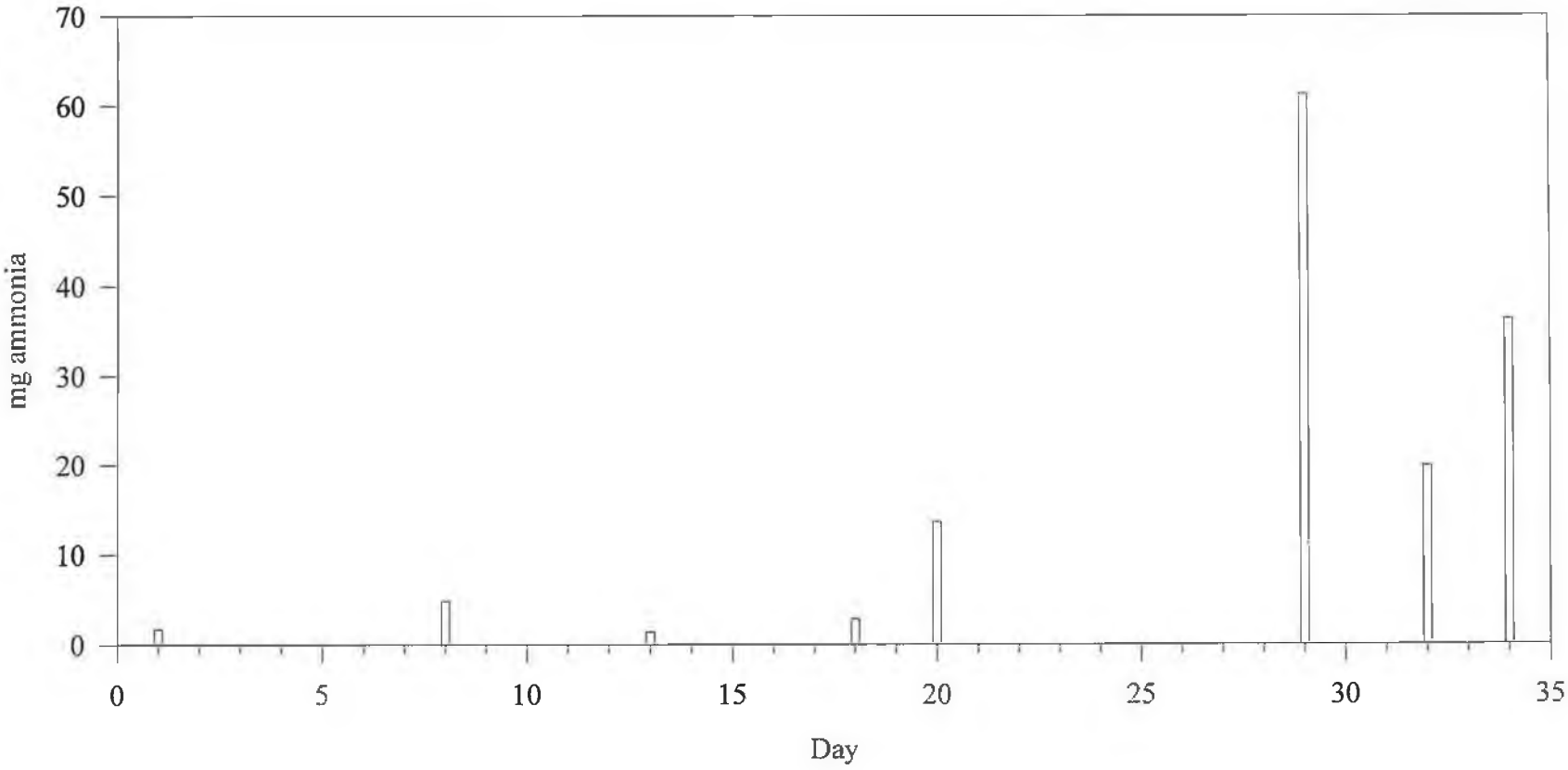


Table 11 Ammonia removal by the biofilter during stage 1

Day	AMMONIA SUPPLIED		UNTREATED AMMONIA THAT EMERGED FROM THE FILTER			Nitrates in percolate	pH of percolate	Removal Efficiency (%)
	NH_3 in (mg)	Mass load g NH_3 Kg^{-1} peat h^{-1}	NH_3 in outlet gas stream (mg)	NH_3 in percolate				
				Conc. (mg L^{-1})	Amount (mg)			
1	1.70	0.0005	0.58	ND	ND	ND	ND	66.7
8	4.80	0.0005	1.58	ND	ND	ND	ND	67.3
13	1.40	0.0010	0.53	378	5.3	+	7.42	61.7
18	2.80	0.0007	0.96	ND	ND	ND	ND	65.3
20	13.60	0.0016	4.72	546	5.46	+	8.00	65.3
29	61.25	0.0077	1.85	135	22.95	+	7.53	97.0
32	19.80	0.0025	2.49	157	3.45	+	6.21	86.7
34	36.15	0.0039	1.54	150	0.45	+	6.73	95.7

ND = not determined – there was no percolate at these times

3.2.2 Microbiology of the packing material during stage 1

The microbiology of the biofilter was studied in order to investigate the relative numbers of bacteria and fungi in the system (expressed as colony forming units (cfu) $gram^{-1}$ peat) together with their role in the removal of ammonia. Many microorganisms are considered to play an important role in nitrogen metabolism, however nitrification is a critical step in ammonia metabolism and so in investigating the numbers of bacteria, the numbers of nitrifying bacteria were also examined. The microbiology of the native peat was first examined to determine whether the indigenous population was suitable or whether inoculation of the peat would be necessary. Inoculation of the peat was found to be necessary.

3.2.2.1 Inoculation of the peat

No nitrifiers were detected on the native peat (Table 12). As the presence of these microorganisms was deemed necessary for the optimal operation of the biofilter, a sample of nitrifying activated sludge was used to inoculate the biofilter. The microbiology of the peat before inoculation, the microbiology of the activated sludge used as the inoculum and the estimated microbial population on the peat following inoculation are presented in Table 12. The estimated numbers of microorganisms present on the peat at the beginning of stage 1 were calculated by combining the numbers present on the native peat and those present in the inoculum, bearing in mind that 200 ml of the activated sludge was used to inoculate 8 Kg of peat (Table 12).

Estimated microbial population on the peat at the beginning of stage 1:

Estimated no. inoculated onto the peat from the activated sludge

= Activated sludge (cfu ml^{-1}) x vol. Inoculum (200ml) ÷ mass peat (8000g)

= cfu g^{-1} peat

Total cfu g^{-1} peat = Native peat (cfu g^{-1} peat) + Activated sludge (cfu g^{-1} peat)

Percentage recovery of cells following inoculation

Twenty four hours following inoculation, the numbers of microorganisms in the biofilter were investigated. Not all of the estimated populations were detected in any case. The numbers detected varied with the microbial population and the location in the biofilter examined.

Bacteria

Following inoculation of the peat with the activated sludge, the numbers of bacteria were estimated to increase 100 fold. However, no more than 72 % of these bacteria could be detected in the system 24 hours later (Table 13). The greatest percentage recovery was at Port B, which was in the middle of the biofilter. Fewest bacteria were enumerated from the bottom of the biofilter (Port C) and only 33 % were recovered at the top of the biofilter (Port A). These values probably reflected the degree to which the inoculum percolated through the system.

Nitrifiers

Prior to inoculation there were no nitrifiers on the peat. Nitrifiers, 2×10^5 cfu ml^{-1}, were present in the activated sludge inoculum. Again, as with the bacterial population not all of these organisms were detected in the system when examined 24 hours later. The highest number of nitrifiers, 80%, were recovered from the bottom of the biofilter, Port C, where the lowest number of total bacteria had been detected (Table 13). This suggested that the nitrifying population was not uniformly dispersed throughout the activated sludge. The numbers of nitrifiers recovered increased with increasing distance from the point of inoculation.

Fungi

A relatively high number of fungi were detected on the native peat. Similarly, a high number of fungi were found to be present in the sample of activated sludge. In general the level of recovery of the fungi from the system was high and better than that of the bacterial populations. The numbers of fungi varied throughout the biofilter.

The highest recovery of fungal cells occurred at sample port A, where there was an 87.5 % recovery of cells. Recovery was 39 % and 61 % respectively at sample ports B and C (Table 13). This result again indicated that the fungal population was not evenly distributed throughout the inoculum.

3.2.2.2 Microbial changes on the peat during stage 1

The bacterial and fungal populations were monitored periodically during stage 1 in order to determine their response to ammonia.

Bacteria

The numbers of bacteria detected in the system 24 hours after inoculation, as previously stated are outlined in Table 13. They are also represented in Fig. 14 at time 0. At this point ammonia was introduced to the system. When the system was sampled at day 7, the cell numbers at sample port A had decreased from 59.0 ± 0.8 x 10^4 cfu g^{-1} peat (immediately after inoculation) to 8.9 ± 1.6 x 10^4 cfu g^{-1} peat. Similarly, at sample port B, the cell numbers decreased from 127.0 ±17.0 x 10^4 cfu g^{-1} peat (immediately after inoculation) to 33.0 ± 4.7 x 10^4 cfu g^{-1} peat. The numbers of bacterial cells at sample port C, closest to the ammonia inlet increased from 12.4 ± 3.0 x 10^4 cfu g^{-1} peat (immediately after inoculation) to 31.0 ± 2.5 x 10^4 cfu g^{-1} peat. The cell counts at sample port C were relatively constant throughout stage 1 and did not increase above 31.0 ± 2.5 x 10^4 cfu g^{-1} peat.

The numbers of bacteria detected at Ports A and B varied during stage 1. Bacterial cell numbers varied between 8.9 ± 1.6 x 10^4 cfu g^{-1} peat and 42.0 ± 4.5 x 10^4 cfu g^{-1} peat at sample port A. At sample port B the cell numbers ranged from 23.0 ± 1.4 x 10^4 cfu g^{-1} peat to 103.0 ± 12.0 x 10^4 cfu g^{-1} peat.

By the end of stage 1 the bacterial counts at all three ports was approximately 21.5 ± 0.9 x 10^4 cfu g^{-1} peat (Fig. 14).

Nitrifiers

Nitrifiers at sample port A decreased steadily from 6.0 x 10^2 cfu g^{-1} peat (count following inoculation, before ammonia was supplied) to 0.3 x 10^2 cfu g^{-1} peat. The general trend at sample port B also indicated that the nitrifier counts decreased during stage 1 to a final value of 2.4 x 10^2 cfu g^{-1} peat on day 27. However at sample Port C the numbers of nitrifiers detected were highest. This sample port was closest to the ammonia inlet and in general the numbers of nitrifiers detected increased with increasing proximity to the gas inlet. The numbers of nitrifiers detected at Port C fluctuated. On day 7 the nitrifiers increased from 40.0 x 10^2 cfu g^{-1} peat (count

following inoculation, before ammonia was supplied) to 140.0 x 10^2 cfu g $peat^{-1}$ but then decreased 150-fold to 0.9 x 10^2 cfu g $peat^{-1}$ by day 15. At the end of stage 1 the cell count was 23 x 10^2 cfu g $peat^{-1}$ (Fig. 15). In general the numbers of nitrifiers at the end of stage 1 were lower than at the beginning, however the nitrifiers did survive the biofilter environment during stage 1.

Fungi

In general, the fungal population survived well in the biofilter during stage 1. The numbers detected varied with the sample port. However, at port A, the number of fungi decreased rapidly from 224.0 ± 8.8 x 10^3 cfu g^{-1} peat (count following inoculation, before ammonia was supplied) to 64.0 ± 2.3 x 10^3 cfu g^{-1} peat by day 7. The numbers of fungal cells detected at this sample port continued to decrease but more gradually for the remainder of stage 1 to 28.0 ± 4.1 x 10^3 cfu g^{-1} peat by day 27.

At sample port B the cell numbers increased to a maximum of 270.0 ± 15.0 x 10^3 cfu g^{-1} peat on day 15 but decreased 3-fold to 94.0 ± 4.3 x 10^3 cfu g^{-1} peat by the end of stage 1. Initially the cells decreased at sample port C from 156.0 ± 3.3 x 10^3 cfu g^{-1} peat (count following inoculation before ammonia was supplied) to 39.0 ± 3.5 x 10^3 cfu g^{-1} peat but increased to a maximum of 360.0 ± 21.0 x 10^3 cfu g^{-1} peat by day 27 (Fig. 16).

The results suggested that while the numbers of fungi did survive in the system, they survived best closest to the ammonia inlet.

Fig. 14 Bacterial enumeration of the peat during stage 1 of biofilter operation

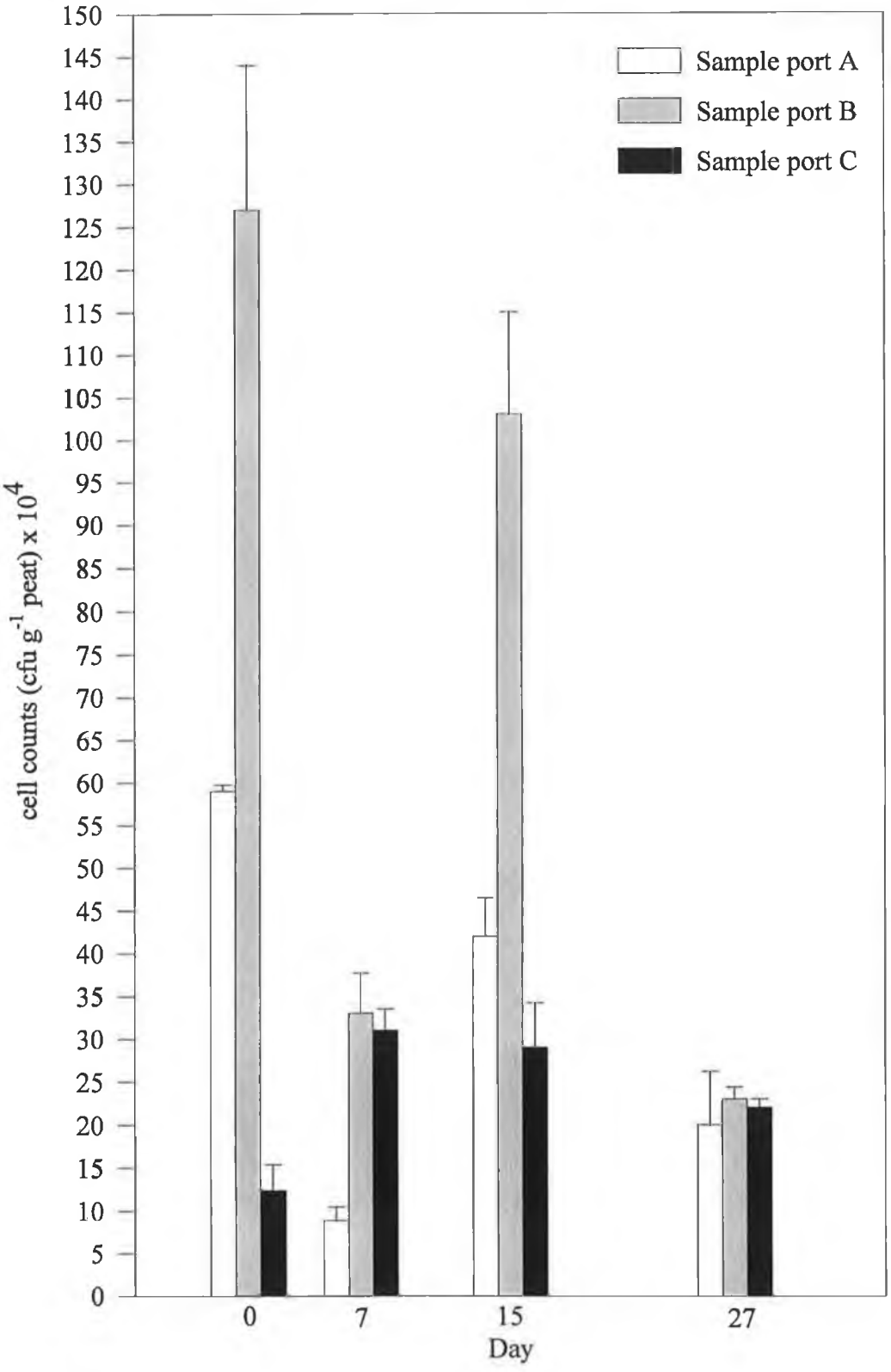


Fig. 15 Nitrifier enumeration of the peat during stage 1 of biofilter operation

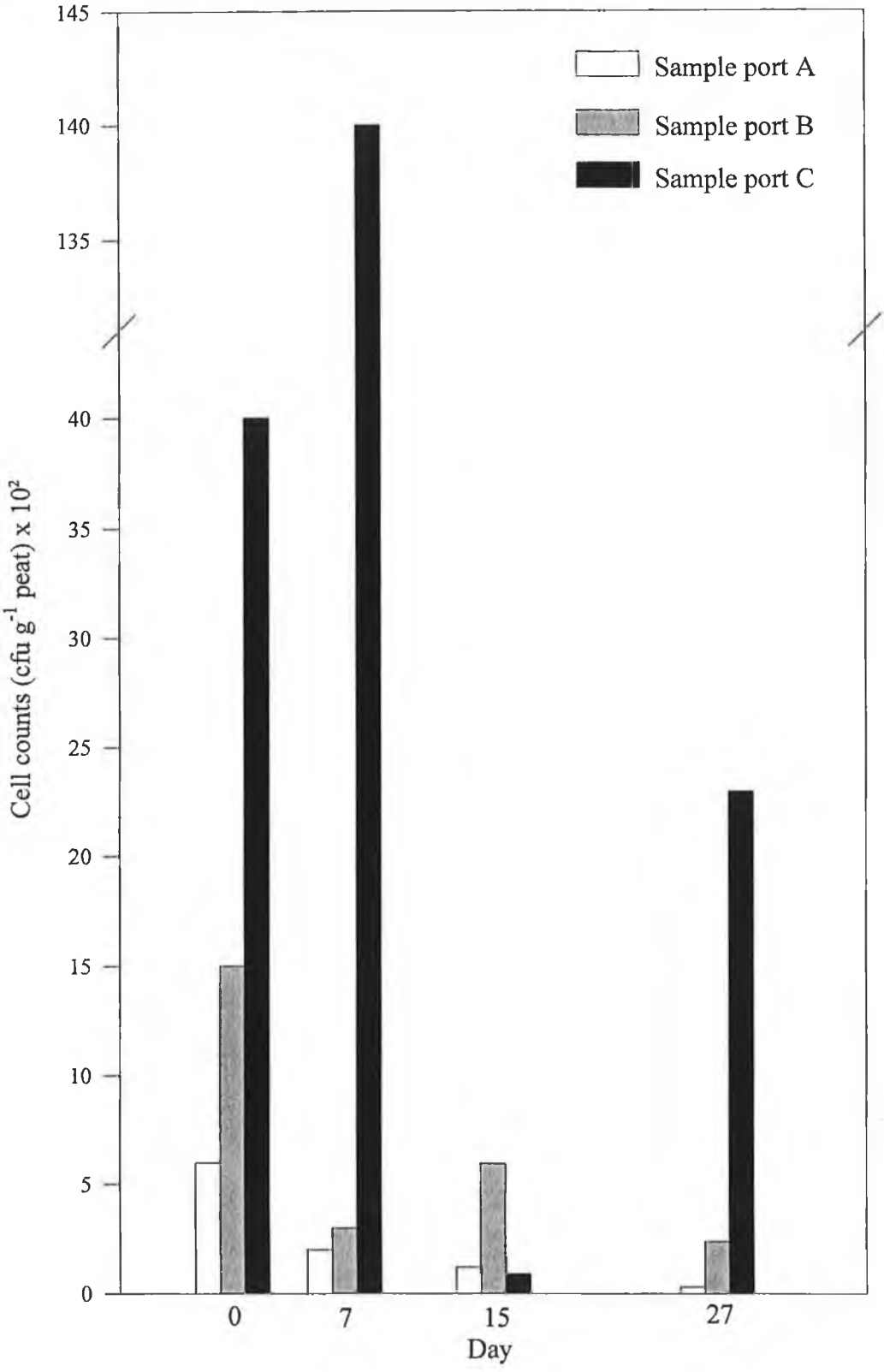
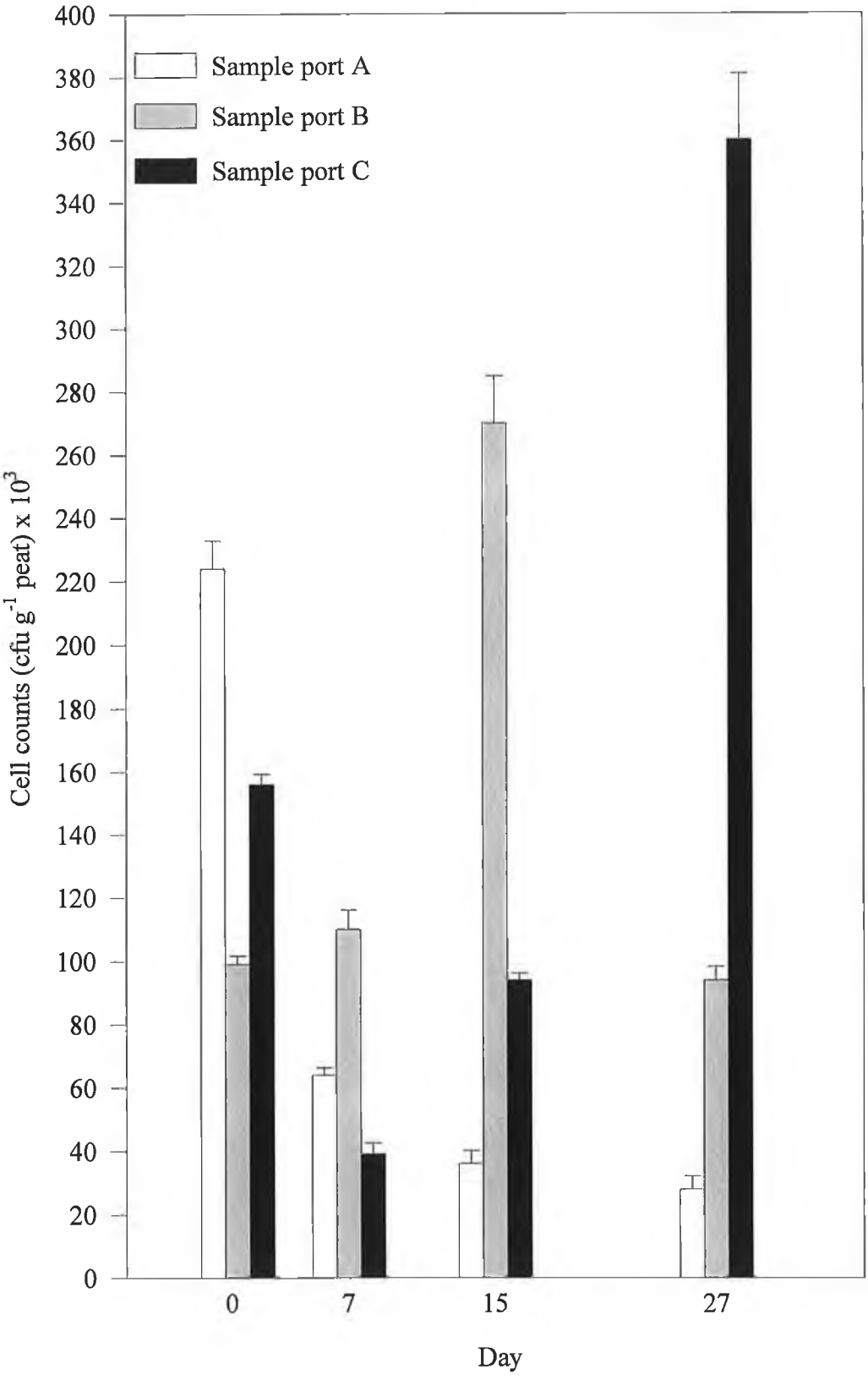


Fig. 16 Fungal enumeration of the peat during stage 1 of biofilter operation



3.3 Stage 2

While the biofilter coped well with the removal of ammonia during stage 1, it was of interest to determine how the biofilter would respond to higher concentrations of ammonia. This stage of investigation extended from day 51 to day 112. As with stage 1, the biofilter was operated in discontinuous mode and the removal of ammonia together with the microbiology of the system was monitored for that period of time.

3.3.1 Ammonia removal

The amount of ammonia supplied to the biofilter during each run of stage 2 is illustrated in Fig. 16. The inlet concentrations and/or the run times were gradually increased from stage 1 values in order to increase the amount of ammonia supplied in any one run and to minimise shock-loading.

On day 51, the first run during stage 2, 93.2 mg ammonia was supplied to the system at a mass load of 0.0093 g NH_3 Kg^{-1} peat d^{-1} (Table 14). The ammonia supplied was gradually increased to 2246.4 mg by day 63 (Fig. 17). This corresponded to a mass load of 0.088 g NH_3 Kg^{-1} peat d^{-1} (Table 14). For the remainder of stage 2 the ammonia supplied in each run varied between 777.6 mg and 5153.4 mg (Fig. 17). Mass loads ranged from 0.068 g NH_3 Kg^{-1} peat d^{-1} and 0.122 g NH_3 Kg $peat^{-1}$ d^{-1} (Table 14). The largest amount of ammonia was supplied on day 115 when 5153.4 mg ammonia was supplied at a mass load of 0.122 g NH_3 Kg^{-1} peat d^{-1}. Although the amount supplied was significantly higher than amounts supplied before and after that day, the mass load was similar to those applied to the peat bed on days 69, 70, 112 and 151 when the respective amounts supplied were 1824.0 mg, 4108.0 mg, 3318.0 mg and 3440.0 mg. The removal efficiency was not affected by increasing the amount of ammonia supplied.

On day 51 the removal efficiency was 94.2 %, when 5.4 mg of the inlet ammonia emerged in the outlet gas stream. Although, the amount of ammonia supplied and hence the mass load increased to 209.3 mg and 0.021 g NH_3 Kg^{-1} peat d^{-1} respectively on day 55, the removal efficiency also increased to 98.5 % as only 3.11 mg ammonia emerged in the outlet air. From day 57 to day 182 ammonia in the outlet air stream varied from 5.14 mg to 81.40 mg and the removal efficiency fluctuated between 97.6 % and 99.6 %. On day 185, the final day of stage 2, even

though the ammonia supplied was comparable to previous supplies (3024.0 mg at a mass load of 0.095 g NH_3 Kg^{-1} peat d^{-1}), 149.26 mg emerged in the outlet air and the efficiency decreased to 95.0 % (Table 14).

The pH of the percolate varied between pH 5.95 and pH 8.35. Ammonia concentrations in the percolate increased gradually from 242 mg L^{-1} on day 51 to 2080.0 mg L^{-1} on day 116. Between day 116 and day 137, the concentrations were relatively constant 2010.0 ± 70.0 mg L^{-1}. There was a significant increase on day 162 when the concentration of ammonia increased to 8510.0 mg L^{-1}. At this time the packing material had been disturbed. There was a threat of holes developing which necessitated a gently mixing of the peat. This mixing may have caused the release of ammonia which had accumulated in the system. The concentration of ammonia in the percolate decreased by 50 % again by day 181. The amounts of ammonia contained in the percolate were dependent on the volume of run-off and therefore varied from 3.9 mg to 322.5 mg ammonia. In all cases the amounts of ammonia detected in the percolate during stage 2 were small compared with the amounts of ammonia in the inlet stream.

Nitrate concentrations in the percolate followed the same trend as the ammonia concentrations. During the early part of stage 2 nitrite and/or nitrates in the percolate were detected using the diphenylamine indicator, however from day 63 onwards the concentration of nitrate was measured with the nitrate ion specific electrode. Prior to day 63 nitrites and/or nitrates were present in the percolate in all cases of percolate analysis. The concentration of nitrate reached a maximum of 24900 mg L^{-1} on day 162 and decreased by almost a third on day 181. The amounts of nitrate in the system ranged from 4.24 mg to 897.60 mg (Table 14). There was a steady increase in the overall levels of nitrate in the percolate indicating that nitrification continued throughout stage 2 even as high levels of ammonia were supplied to the biofilter.

Fig. 17 Ammonia supplied to the biofilter during stage 2 of operation

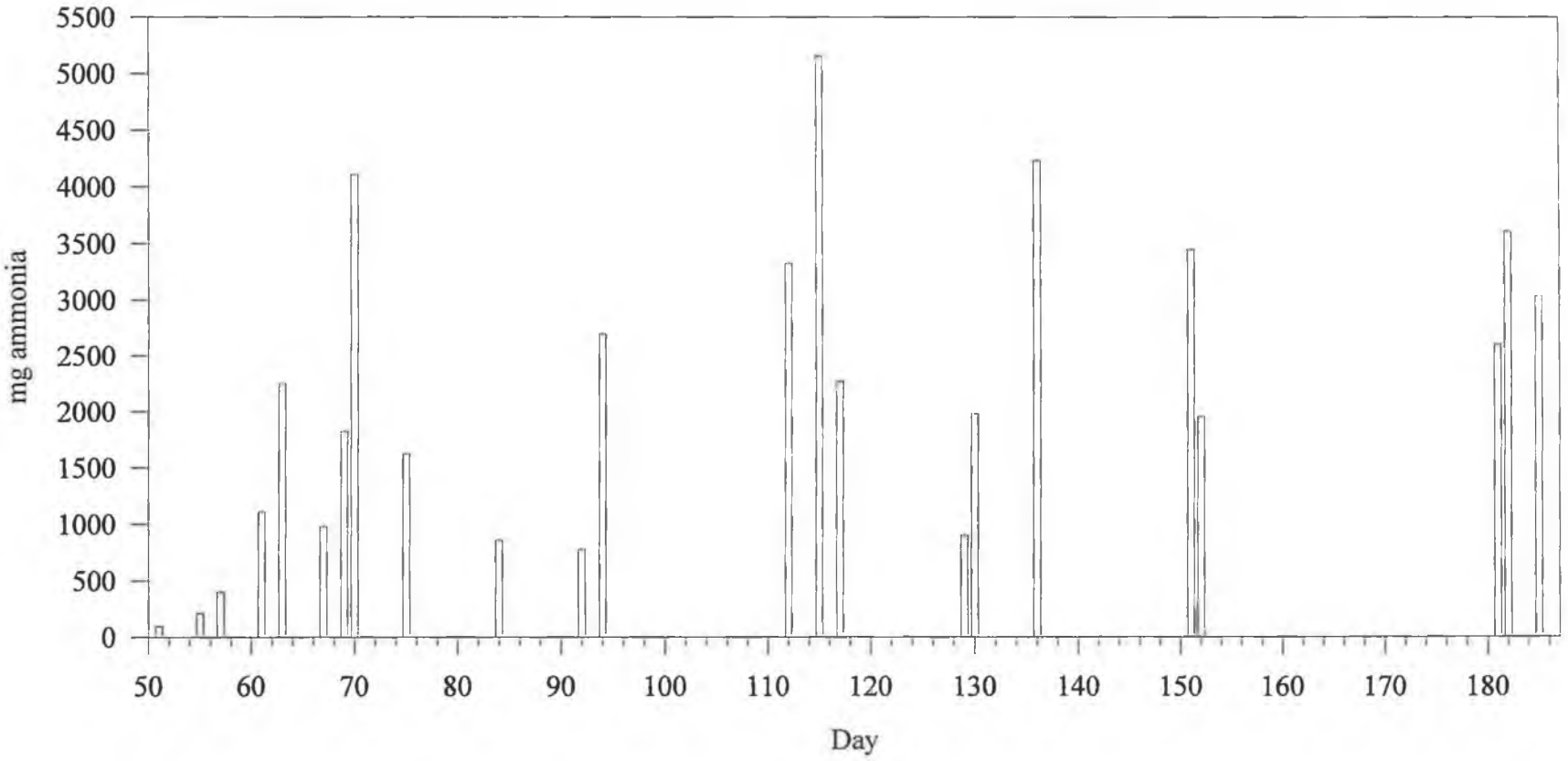


Table 14 Ammonia removal by the biofilter during stage 2

Day	AMMONIA SUPPLIED		UNTREATED AMMONIA THAT EMERGED FROM THE FILTER					pH of percolate	Removal Efficiency (%)
	NH_3 in (mg)	Mass load g NH_3 Kg^{-1} peat h^{-1}	NH_3 in outlet gas stream (mg)	NH_3 in percolate		NO_3 in percolate			
				Conc. (mg L^{-1})	Amount (mg)	Conc. (mg L^{-1})	Amount (mg)		
51	93.2	0.0093	5.40	242	18.15	ND	ND	6.49	94.2
55	209.3	0.021	3.11	379	30.32	ND	ND	5.95	98.5
57	399.6	0.025	5.14	431	3.88	ND	ND	8.05	98.8
61	1109.2	0.032	11.70	433	64.95	ND	ND	6.15	98.9
63	2246.4	0.088	34.66	500	5.00	424	4.24	7.54	98.8
67	980.1	0.082	15.40	862	67.20	2995	233.6	6.94	98.4
69	1824.0	0.114	13.68	ND	ND	ND	ND	ND	99.3
70	4108.0	0.120	14.90	ND	ND	ND	ND	ND	99.6
75	1624.0	0.087	24.85	1140	11.40	4210	4.21	7.56	98.5
84	858.6	0.072	11.20	ND	ND	ND	ND	ND	98.7
92	777.6	0.065	3.24	ND	ND	ND	ND	ND	99.6
94	2691.0	0.104	37.44	1190	59.50	2500	125	7.85	98.6
112	3318.0	0.118	25.20	ND	ND	ND	ND	ND	99.2

Table 14 Ammonia removal by the biofilter during stage 2 (ctnd.)

Day	AMMONIA SUPPLIED		UNTREATED AMMONIA THAT EMERGED FROM THE FILTER			NO_3 in percolate		pH of percolate	Removal Efficiency (%)
	NH_3 in (mg)	Mass load g NH_3 Kg^{-1} peat h^{-1}	NH_3 in outlet gas stream (mg)	NH_3 in percolate					
				Conc. (mg L^{-1})	Amount (mg)	Conc. (mg L^{-1})	Amount (mg)		
115	5153.4	0.122	49.70	ND	ND	ND	ND	ND	98.9
116	No run	No run	No run	2080	62.40	5870	176.1	8.35	No run
117	2268.0	0.095	17.10	ND	ND	ND	ND	ND	99.2
129	900.0	0.090	2.68	2007	124.40	5979	370.7	7.31	99.7
130	1980.0	0.080	20.35	2070	14.50	6180	43.26	7.59	99.0
136	4224.0	0.096	35.02	1750	12.25	5230	36.6	7.72	99.2
137	No run	No run	No run	2150	322.50	5000	750	7.62	No run
151	3440.0	0.120	81.40	ND	ND	ND	ND	ND	97.6
152	1950.0	0.068	20.60	ND	ND	ND	ND	ND	98.9
162	No run	No run	No run	8510	127.65	24900	373.5	7.31	No run
181	2592.0	0.081	39.70	4130	210.63	17600	897.6	7.28	98.5
182	3596.0	0.100	16.70	ND	ND	ND	ND	ND	99.6
185	3024.0	0.095	149.26	ND	ND	ND	ND	ND	95.0

3.3.2 Microbiology of the packing material during stage 2

As was the case with stage 1, the numbers of total bacteria, nitrifying bacteria and fungi were monitored during stage 2.

Bacteria

The numbers of bacteria in the biofilter fluctuated during stage 2 at sample ports A and B. Between days 51 and 141, the mean numbers of bacteria at these two sample points were $2.5 \pm 0.5 \times 10^4$ cfu g^{-1} peat and $14.9 \pm 2.5 \times 10^4$ cfu g^{-1} peat respectively, which was slightly lower than counts during stage 1 (Fig. 18).

At sample port C however, the numbers of bacteria during stage 2 were stable until day 105 at $19.7 \pm 3.4 \times 10^4$ cfu g^{-1} peat, after which they increased steadily and reached a peak of $680.0 \pm 83.0 \times 10^4$ cfu g^{-1} peat on day 141 (Fig. 18).

At all three sample ports the cell counts decreased between day 141 and 176. The decrease coincided with a period when no ammonia was supplied to the system. (The last supply of ammonia before the microbial enumeration was conducted was day 152, which implied a 24 d shut-down period to the microbes (Fig. 18).

Nitrifiers

Initially the nitrifiers numbers at sample port A were similar to numbers during stage 1, ranging from 0.8×10^2 cfu g^{-1} peat – 2.0×10^2 cfu g^{-1} peat but by day 62 the cell count had decreased. In some cases no nitrifiers could be detected. Between days 62 and 176 the mean number of nitrifiers was $0.21 \pm 0.08 \times 10^2$ cfu g^{-1} peat (Fig. 19).

From day 50 to day 143 the cell numbers at sample port B were comparable to counts during stage 1, varying from 0.15×10^2 cfu g^{-1} peat – 3.4×10^2 cfu g^{-1} peat. Maximum growth occurred between day 143 and day 176 when the cell counts increased to 8.6×10^2 cfu g^{-1} peat (Fig. 19).

At sample port C, the nitrifier numbers fluctuated between 0.14×10^2 cfu g^{-1} peat to 19.0×10^2 cells g^{-1} peat between day 50 and day 143, which were comparable to cell numbers during stage 1. The cell counts increased significantly between day 143 and day 176, when the cell numbers increased from 0.8×10^2 cfu g^{-1} peat to 290.0×10^2 cfu g^{-1} peat (Fig. 19).

This increase in cell numbers, also mirrored at sample port B corresponded to the 24 day shut-down period, during which time there was no ammonia supply to the

system. In general the numbers of nitrifiers were greater closest to the supply of ammonia.

Fungi

Between day 50 and day 86, the fungal numbers at sample port A were similar to stage 1 counts. The counts ranged from 22.0 ± 0.56 x 10^3 cfu g^{-1} peat to 70 ± 3.7 x 10^3 cfu g^{-1} peat. By day 116 the numbers had decreased 10-fold to 5.7 ± 0.16 x 10^3 cfu g^{-1} peat. Cell growth remained below 10 x 10^3 cells g^{-1} peat for the rest of stage 2 (Fig. 20).

Initially the fungal count at sample port B was comparable to stage 1 values but there was a steady decline in numbers between day 50 and day 116. The cell numbers decreased from 260.0 ± 10.0 x 10^3 cfu g^{-1} peat (day 50) to 4.7 ± 0.5 x 10^3 cells g^{-1} peat (day 116). For the remainder of stage 2 the fungal counts remained below 10.0 x 10^3 cells g^{-1} peat (Fig. 20).

At sample port C the fungi fluctuated between 7.0 ± 2.7 x 10^3 cfu g^{-1} peat and 240.0 ± 17.0 x 10^3 cells g^{-1} peat throughout stage 2 (Fig. 20). The counts were similar to those of stage 1.

The general trend indicated that the fungi could not survive in the biofilter under stage 2 conditions.

Fig. 18 Bacterial Enumeration of the Peat during stage 2 of Biofilter Operation

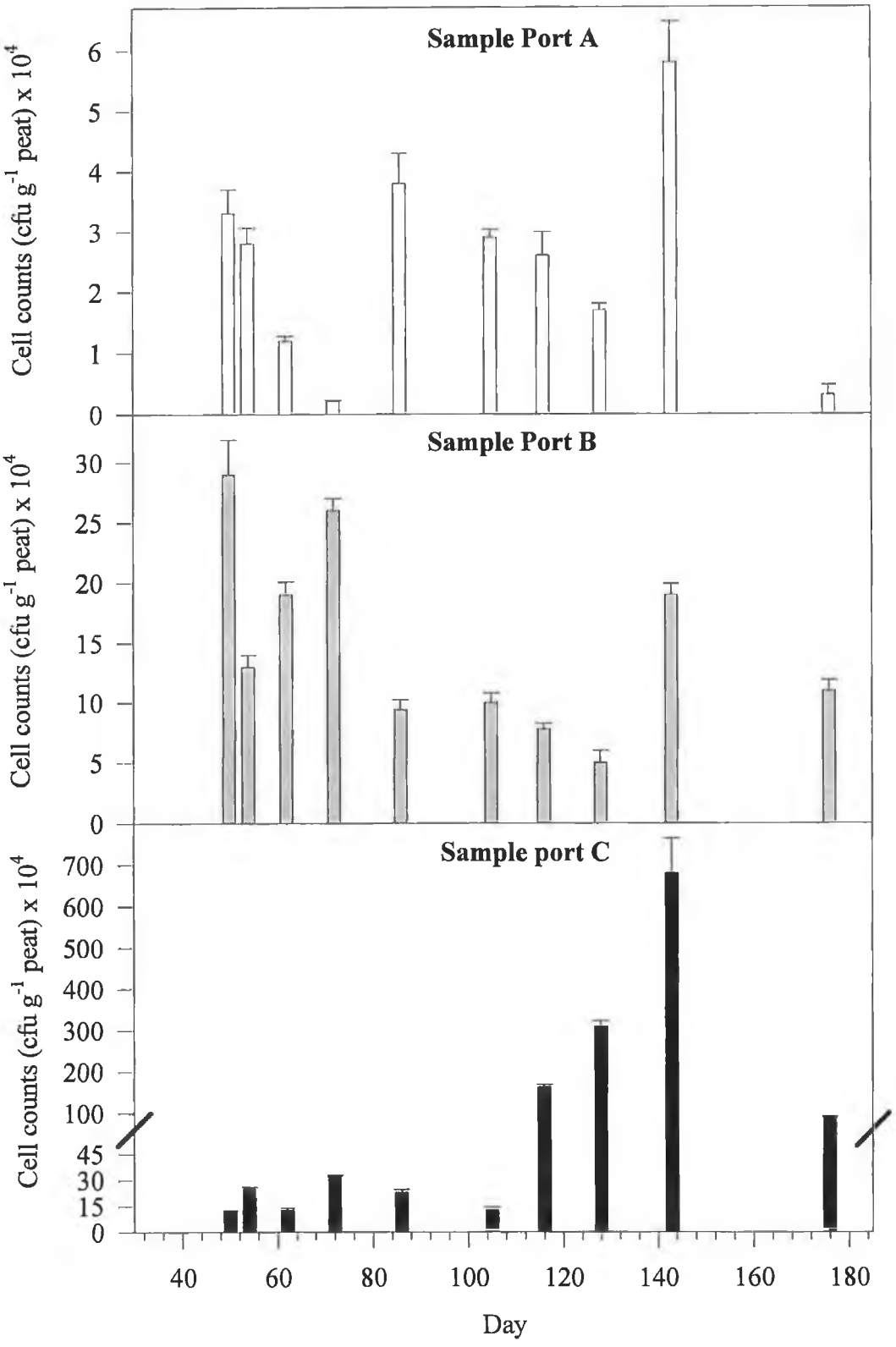


Fig. 19 Nitrifier Enumeration on the Peat during Stage 2 of Biofilter Operation

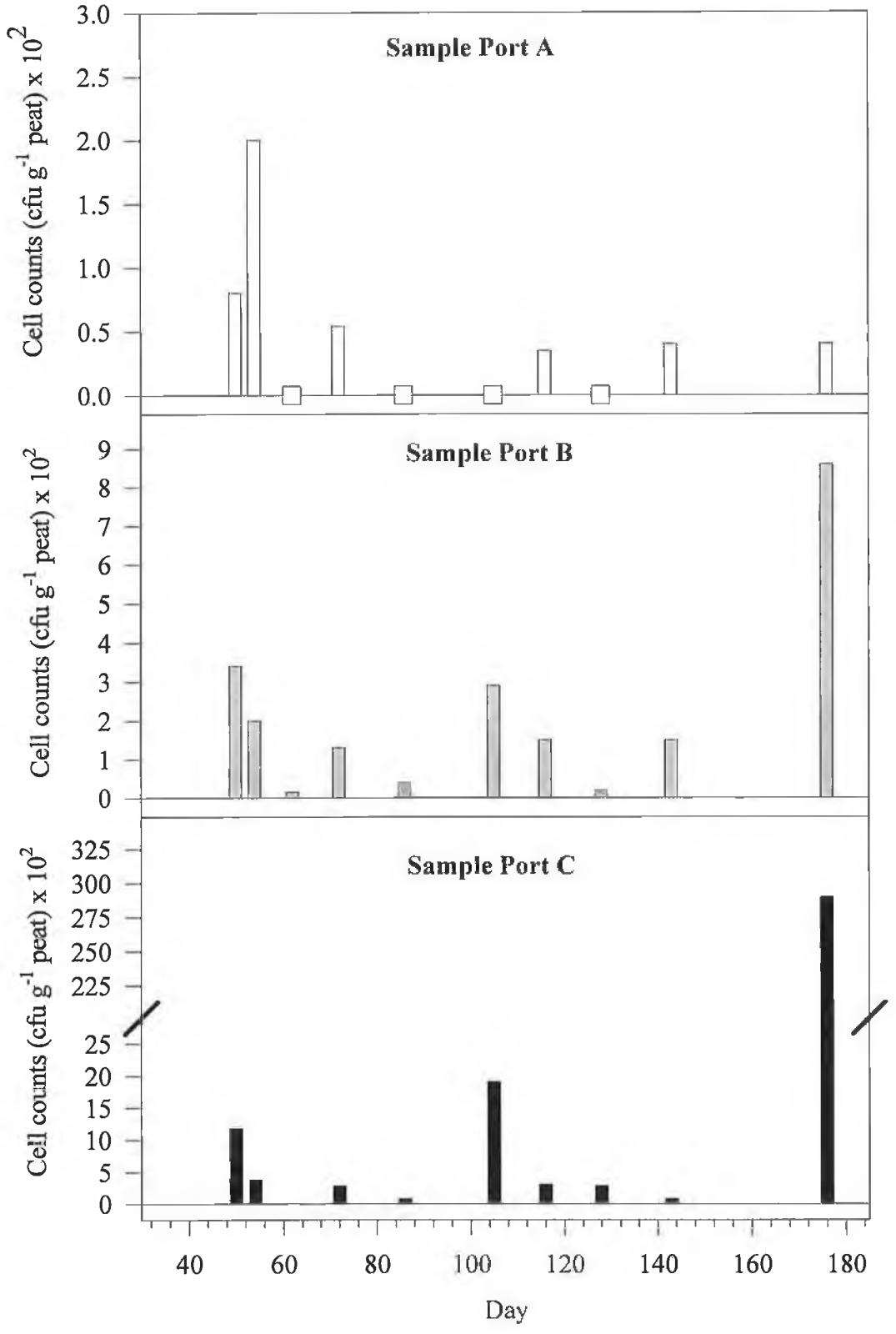
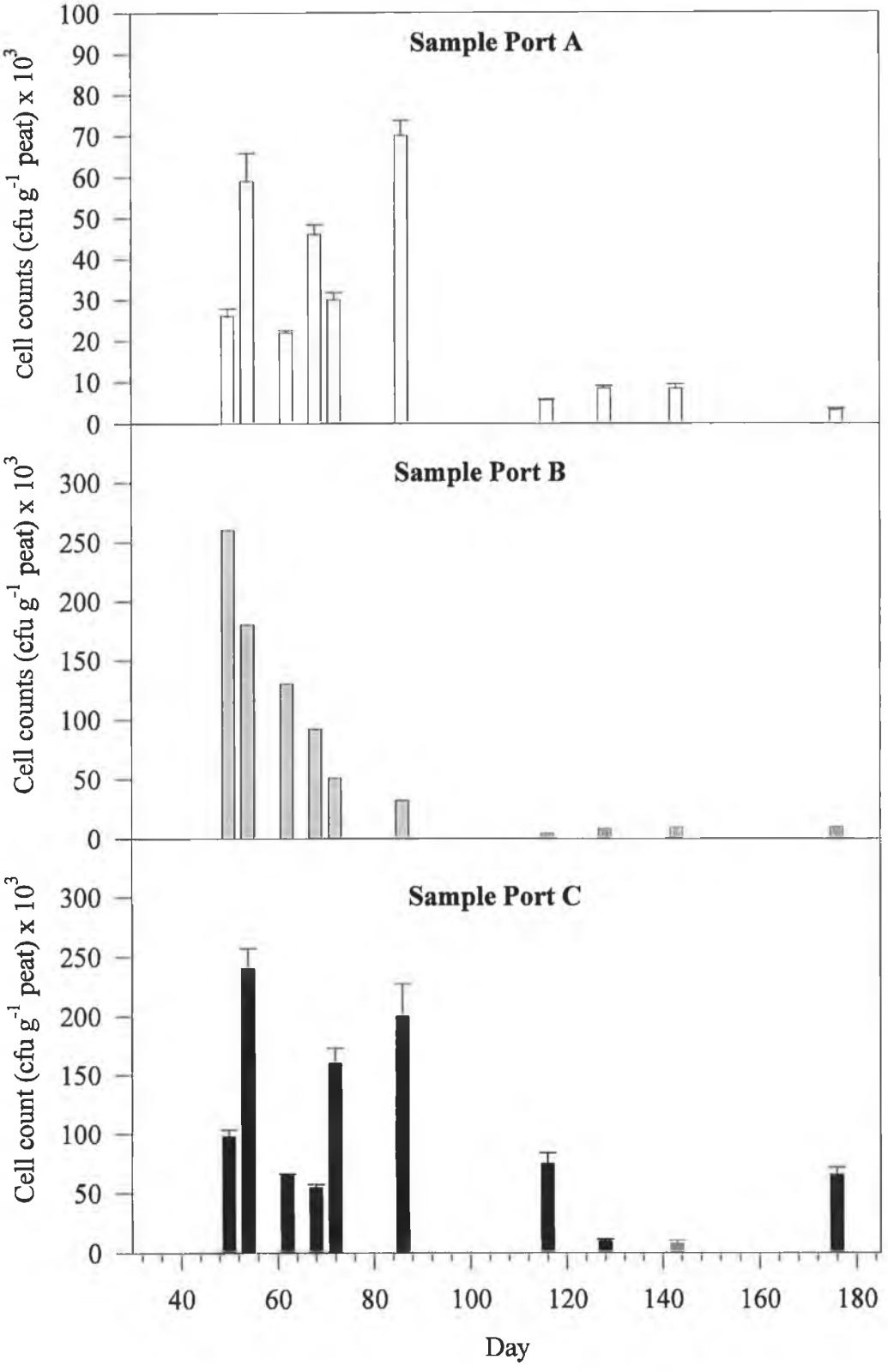


Fig. 20 Fungal Enumeration of the Peat during Stage 2 of Biofilter Operation



3.4 Stage 3

During the final stage of operation, the biofilter was challenged with yet higher loadings of ammonia. Ammonia removal and the microbiology of the system were monitored. The physical structure of the packing material was also observed during this stage.

3.4.1 Ammonia removal

The amount of ammonia supplied to the biofilter during each run of stage 3 is represented in Figure 21. The amount of ammonia supplied was increased significantly from 3024 mg on day 185 (the end of stage 2) to 8316 mg on day 186 (first day of stage 3). This corresponded to an increase in mass load from 0.095 g NH_3 Kg^{-1} peat to 0.3 g NH_3 Kg^{-1} peat. No adverse affects resulted from the increase in ammonia supply; the removal efficiency was 99.8 % as only 15.1 mg of the inlet ammonia emerged in the outlet stream (Table 15).

From day 186 to day 235, the average amount of ammonia supplied in each run was 10524 ± 596 mg (Fig. 21). The mass loads varied from 0.24 g NH_3 Kg^{-1} peat to 0.380 g NH_3 Kg^{-1} peat (Table 15). The highest amount of ammonia supplied to the peat bed was 15255 mg on day 234 (Fig. 21).

The amount of ammonia supplied in each run was reduced between day 244 and day 276 because when the higher amounts were supplied the removal efficiency fluctuated more pre-dominantly than during stage 2 and the general trend was a decrease in efficiency. As already stated, the removal efficiency at the beginning of stage 3 was 99.8 % but it decreased to 92.6 % by day 201 when 691.4 mg of the inlet ammonia emerged in the exhaust air. On day 207, although a similar amount of ammonia was supplied to the filter, the removal efficiency recovered to 97.0 % but by day 235, the efficiency had decreased again to 92.6 %. Between day 244 and day 276 1987 mg – 5789 mg (except for day 247, when 10735 mg was supplied) (Fig. 20). Although the amount of ammonia supplied was reduced, the mass loads remained high at 0.230 – 0.360 g NH_3 Kg^{-1} peat (Table 15). At the lower amounts of ammonia supplied the removal efficiency initially increased but quickly decreased again and fluctuated between 92.8 % and 98.9 % for the remainder of that period.

From day 276 until the end of stage 3, the inlet ammonia was increased again to 6264 mg – 10714 mg (Fig. 20). Although only 6264 mg ammonia was supplied on

day 293 the corresponding mass load was 0.390 g NH_3 Kg^{-1} peat, which was the highest load applied to the peat bed. The removal efficiency continued to decrease and by the end of stage 3 the removal efficiency was 89.1 % (Table 15). Throughout stage 3 the amount of ammonia that emerged in the outlet stream varied from 15.1 mg when 8316 mg ammonia was supplied to the biofilter to 873.6 when 10735 mg was supplied.

The pH of the percolate in general was slightly higher than stage 2 values, it varied between pH 7.62 and pH 8.98. Ammonia concentration was constant at 2500 ± 150 mg L^{-1} until day 215, after which it gradually decreased to 462 mg L^{-1} by the end of stage 3. There was very little percolate after day 247 although water was regularly added to the system. The amount of untreated ammonia that emerged in the percolate varied from 15.5 mg (day 272) to 227.7 mg (day 201). Nitrate concentrations in the percolate also decreased during stage 3 to a minimum of 625 mg L^{-1} on day 297. The amount of nitrate ranged from 34.4 mg (day 297) to 919.6 (day 201) (Table 15).

The packing material compacted under the harsh conditions supplied to the biofilter in stage 3.

Fig. 21 Ammonia supplied to the biofilter during stage 3 of operation

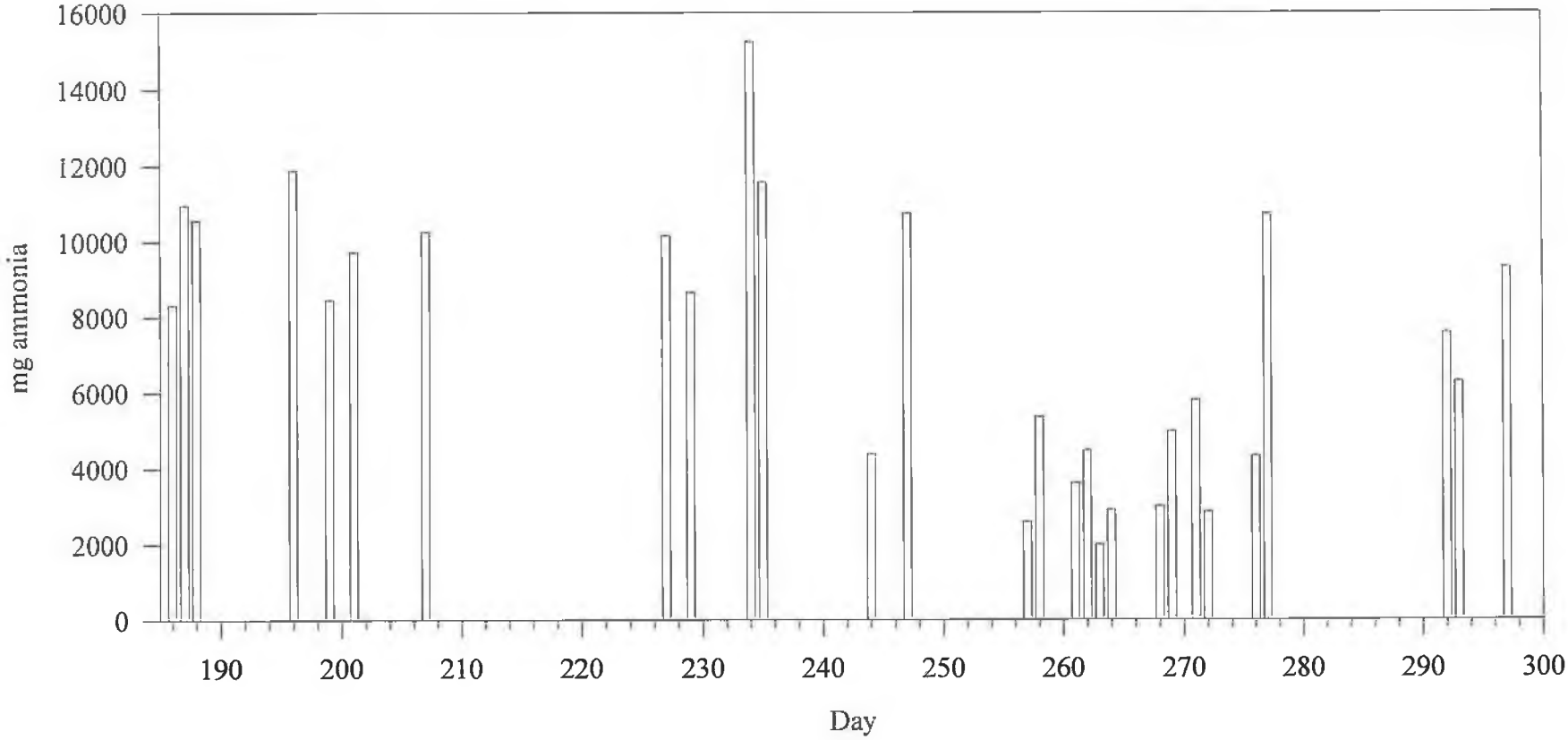


Table 15 Ammonia removal by the biofilter during stage 3

Day	AMMONIA SUPPLIED		UNTREATED AMMONIA THAT EMERGED FROM THE FILTER					pH of percolate	Removal Efficiency (%)
	NH_3 in (mg)	Mass load g NH_3 Kg^{-1} peat h^{-1}	NH_3 in outlet gas stream (mg)	NH_3 in percolate		$[NO_3]$ in percolate			
				Conc. (mg L^{-1})	Amount (mg)	Conc. (mg L^{-1})	Amount (mg)		
186	8316	0.3	15.1	ND	ND	ND	ND	ND	99.8
187	10962	0.27	66.3	ND	ND	ND	ND	ND	99.4
188	10557	0.31	130.1	2476	123.8	9100	455.0	8.10	98.7
196	11880	0.27	160.7	ND	ND	ND	ND	ND	98.7
199	8456	0.24	176.6	ND	ND	ND	ND	ND	97.9
201	9720	0.30	691.4	2070	227.7	8360	919.6	8.96	92.9
202	No run	No run	No run	2840	28.4	10700	107.0	8.00	No run
207	10260	0.27	309.6	ND	ND	ND	ND	ND	97.0
214	No run	No run	No run	2340	70.2	6000	180.0	8.19	No run
215	No run	No run	No run	2860	28.6	5000	50.0	8.02	No run
227	10152	0.32	313.0	ND	ND	ND	ND	ND	96.9
229	8640	0.32	4.6.8	1450	21.8	2630	394.5	8.98	95.3
234	15255	0.38	510.3	ND	ND	ND	ND	ND	95.4

Table 15 Ammonia removal by the biofilter during stage 3 (ctnd.)

Day	AMMONIA SUPPLIED		UNTREATED AMMONIA THAT EMERGED FROM THE FILTER						
	NH_3 in (mg)	Mass load g NH_3 Kg^{-1} peat h^{-1}	NH_3 in outlet gas stream (mg)	[NH_3] in percolate		[NO_3] in percolate		pH of percolate	Removal Efficiency (%)
				Conc. (mg L^{-1})	Amount (mg)	Conc. (mg L^{-1})	Amount (mg)		
235	11567	0.28	850.0	ND	ND	ND	ND	ND	92.6
244	4376	0.25	128.7	ND	ND	ND	ND	ND	97.1
247	10735	0.29	873.6	745	149.0	1425	285	8.52	91.9
257	2592	0.32	28.1	ND	ND	ND	ND	ND	98.9
258	5346	0.30	216.3	ND	ND	ND	ND	ND	96.0
261	3607	0.23	142.6	ND	ND	ND	ND	ND	96.0
262	4471	0.28	194.4	ND	ND	ND	ND	ND	95.7
263	1987	0.25	137.2	ND	ND	ND	ND	ND	93.1
264	2902	0.28	191.7	ND	ND	ND	ND	ND	93.2
268	2997	0.25	176.6	ND	ND	ND	ND	ND	94.1
269	4968	0.31	341.3	ND	ND	ND	ND	ND	93.1
271	5789	0.36	413.6	ND	ND	ND	ND	ND	92.8

Table 15 Ammonia removal by the biofilter during stage 3 (ctnd.)

Day	AMMONIA SUPPLIED		UNTREATED AMMONIA THAT EMERGED FROM THE FILTER			[NO_3] in percolate		pH of percolate	Removal Efficiency (%)
	NH_3 in (mg)	Mass load g NH_3 Kg^{-1} peat h^{-1}	NH_3 in outlet gas stream (mg)	NH_3 in percolate					
				Conc. (mg L^{-1})	Amount (mg)	Conc. (mg L^{-1})	Amount (mg)		
272	2844	0.36	196.6	1550	15.5	3530	8.47	8.47	93.1
276	4325.4	0.36	202.5	ND	ND	ND	ND	ND	95.3
277	10714	0.33	541.4	ND	ND	ND	ND	ND	95.0
292	7560	0.27	281.6	1080	32.4	1897	56.9	7.62	96.3
293	6264	0.39	648.0	ND	ND	ND	ND	ND	89.7
297	9288	0.35	1015.2	462	25.4	625	34.35	8.48	89.1

ND = not determined. There was no percolate from the system on those days

No run indicates that ammonia was not supplied to the biofilter on those days.

3.4.2 Microbiology of the packing material during stage 3

In order to compensate for the net loss of packing material from the system following sampling, 1.5 Kg peat was added to the top of the biofilter on day 225. Table 16 illustrates the microbiology of a sample of this peat prior to use in the biofilter. As with the peat analysed before stage 1, both bacteria and fungi were detected and no nitrifiers were found. Nitrifying activated sludge was again used to inoculate the biofilter with nitrifying bacteria.

Table 16 Microbial analysis of native peat added to the biofilter during stage 3

	CFU g^{-1} peat
Bacteria	$3.0 \pm 0.5 \times 10^5$
Nitrifiers	0
Fungi	$8.0 \pm 0.9 \times 10^2$

3.4.2.1 Inoculation of the peat

On day 229, the filter was inoculated with nitrifying activated sludge. The microbiology of the peat at sample ports A, B and C before inoculation, the microbiology of the activated sludge used as the inoculum and the estimated microbial population on the peat following inoculation are illustrated in Tables 17, 18 and 19. The estimated population is the sum of the introduced populations, bearing in mind that 750 ml of activated sludge was used to inoculate the peat bed together with the indigenous populations.

Percentage recovery of cells following inoculation

Twenty-four hours after inoculation, the microorganisms in the biofilter were enumerated. The numbers detected varied with the microbial population and the location in the biofilter examined. At the top of the biofilter (Port A) there was a higher percentage recovery of cells from all three populations following inoculation compared with the recovery achieved at that sample port A after inoculation in stage 1. Recovery was greater than 75 % for all three populations and fungal cells achieved the highest recovery of 95 % (Tables 17, 18 and 19).

At sample ports B and C there was less than 1 % recovery of the nitrifier population from the activated sludge. Fungal cells were not detected at these sample ports 24 h after inoculation even though the activated sludge had an indigenous fungal population. In the case of the heterotrophic bacterial populations there was in excess of 100 % recovery of these cells at sample ports B and C. This result suggested that the organisms were actively growing and that the growth of these organisms was greatest at sample port C where there was 1033 % recovery of the organisms as distinct to 110 % recovery at sample port B (Tables 17, 18 and 19).

Table 17 Microbiology of the biofilter before and after inoculation during stage 3 at sample port A

	Count before inoculation (cells g^{-1} peat)	Activated Sludge (cells ml^{-1})	Estimated no. on the peat (cells g^{-1} peat)	Actual no. 24 hrs after inoculation (cells g^{-1} peat)	% Recovery
Bacteria	$2.0 \pm 0.06 \times 10^5$	$8.8 \pm 0.75 \times 10^7$	$8.45 \pm 0.76 \times 10^6$	$65 \pm 3.7 \times 10^5$	77
Nitrifiers	0	3.2×10^6	3.0×10^5	2.5×10^4	83
Fungi	$6.0 \pm 0.52 \times 10^2$	$3.1 \pm 0.5 \times 10^5$	$2.96 \pm 0.61 \times 10^4$	$2.8 \pm 0.07 \times 10^4$	95

Table 18 Microbiology of the biofilter before and after inoculation during stage 3 at sample port B

	Count before inoculation (cells g^{-1} peat)	Activated Sludge (cells ml^{-1})	Estimated no. on the peat (cells g^{-1} peat)	Actual no. 24 hrs after inoculation (cells g^{-1} peat)	% Recovery
Bacteria	$1.0 \pm 0.04 \times 10^7$	$8.8 \pm 0.75 \times 10^7$	$1.825 \pm 0.11 \times 10^7$	$2.0 \pm 0.04 \times 10^7$	110
Nitrifiers	0	3.2×10^6	3.0×10^5	1.8×10^2	< 1
Fungi	0	$3.1 \pm 0.5 \times 10^5$	$2.9 \pm 0.7 \times 10^4$	0	0

Table 19 Microbiology of the biofilter before and after inoculation during stage 3 at sample port C

	Count before inoculation (cells g^{-1} peat)	Activated Sludge (cells ml^{-1})	Estimated no. on the peat (cells g^{-1} peat)	Actual no. 24 hrs after inoculation (cells g^{-1} peat)	% Recovery
Bacteria	$2.4 \pm 0.18 \times 10^6$	$8.8 \pm 0.75 \times 10^7$	$1.065 \pm 0.77 \times 10^7$	$1.1 \pm 0.07 \times 10^8$	1033
Nitrifiers	0	3.2×10^6	3.0×10^5	3.4×10^2	< 1
Fungi	0	$3.1 \pm 0.5 \times 10^5$	$2.9 \pm 0.7 \times 10^4$	0	0

3.4.2.2 Microbial changes on the peat during stage 3

Bacteria

At sample port A bacterial numbers increased 10-fold from $0.32 \pm 0.06 \times 10^4$ cfu g^{-1} peat at the end of stage 2 to $0.5 \pm 0.04 \times 10^5$ cfu g^{-1} peat at the beginning of stage 3. The numbers continued to increase to $450.0 \pm 45.0 \times 10^5$ cfu g^{-1} peat by day 219. There was a decrease in numbers to $2.0 \pm 0.30 \times 10^5$ cfu g^{-1} peat on day 225. This sample was taken following the addition of fresh peat to the biofilter and was a measure of the number of bacteria present on that peat (Fig. 22).
The peat was inoculated with the activated sludge on day 229 and the increase in the numbers of bacteria at sample port A is reflected in the numbers detected on day 230.

The numbers of bacteria continued to increase reaching a maximum number of $1100.0 \pm 71.0 \times 10^5$ cfu g^{-1} peat by day 246 after which time the counts gradually began to decline. At the end of stage 3 the cell count at sample port A was $18.0 \pm 1.5 \times 10^5$ cfu g^{-1} peat (Fig. 22). Although the counts of bacteria were higher than those in stage 2, the bacterial population showed some decline from the middle of stage 3.

Between day 176 (end of stage 1) and day 186 (beginning of stage 2), the bacterial numbers at sample port B increased 3-fold from $11.0 \pm 0.9 \times 10^4$ cfu g^{-1} peat to $3.2 \pm 0.4 \times 10^5$ cfu g^{-1} peat. Bacterial growth increased significantly to $590.0 \pm 18.0 \times 10^5$ cfu g^{-1} peat by day 219 but decreased to $100.0 \pm 4.0 \times 10^5$ cfu g^{-1} peat again on day 225. While the numbers of bacteria did peak at a maximum value of $970 \pm 56 \times 10^5$ cfu g^{-1} peat, on day 260, the bacterial numbers at sample port B remained approximately at this level ($100.0 \pm 4.0 \times 10^5$ cfu g^{-1} peat) for the remainder of stage 3 (Fig. 22).

At sample port C, bacterial numbers continued to decrease from stage 2 values, to $5.0 \pm 0.2 \times 10^4$ cfu g^{-1} peat by day 186. However growth resumed from day 186 and growth in the system was reflected in an increase in bacterial numbers to $140.0 \pm 13.0 \times 10^5$ cfu g^{-1} peat on day 219. With the exception of a decrease in cell numbers

before the biofilter was inoculated with activated sludge and a sharp increase in numbers following inoculation, the numbers of bacteria remained on average at this level in the system for the remainder of stage 3 (Fig. 22).

Nitrifiers

At sample port A, the nitrifiers decreased from 1.7 x 10^2 cfu g^{-1} peat on day 186 to 0.2 x 10^2 cfu g^{-1} peat by day 219. Overall the counts were similar to nitrifier numbers during stage 2. On day 225, after the addition of fresh peat the nitrifier count was 0 as there were no nitrifiers on the native peat. 24 h after inoculation there was 83 % recovery of nitrifiers from the activated sludge which resulted in a cell count of 2500 x 10^2 cfu g^{-1} peat (Table 16). The nitrifiers decreased gradually from 260.0 x 10^2 cfu g^{-1} peat on day 236 until no nitrifiers could be detected on day 286 (Fig. 23).

On day 186, the nitrifier numbers had increased to 2.3 x 10^2 cfu g^{-1} peat from 1.5 x 10^2 cfu g^{-1} peat at the end of stage 2 at sample port B. However, by day 202 no nitrifiers could be detected and were not further detected until the peat was re-inoculated with activated sludge. Immediately after inoculation there were 1.8 x 10^2 nitrifiers g^{-1} peat. The inoculated cells grew under biofilter conditions as the cell numbers increased to 200.0 x 10^2 cfu g^{-1} peat by day 236. Within 10 days, the nitrifiers decreased to 0.2 x 10^2 cfu g^{-1} peat and on day 286 no further nitrifiers could be detected at this sample port (Fig. 23).

The nitrifiers enumerated at sample port C were similar to the counts at the end of stage 2 until day 225. They were present with an average count of 2.5 ± 1.3 x 10^2 cfu g^{-1} peat. Immediately after the peat was inoculated the cell counts were 3.4 x 10^2 cfu g^{-1} peat, which was as a result of 0.11 % recovery of nitrifiers from the sludge (Table 18). By day 236 the nitrifiers had increased to 160.0 x 10^2 cfu g^{-1} peat resembling the pattern at sample port B. Ten days later however, the counts had decreased again to 1.5 x 10^2 cfu g^{-1} peat and continued to decrease until at day 286 no further nitrifiers could be detected (Fig. 23).

Fungi

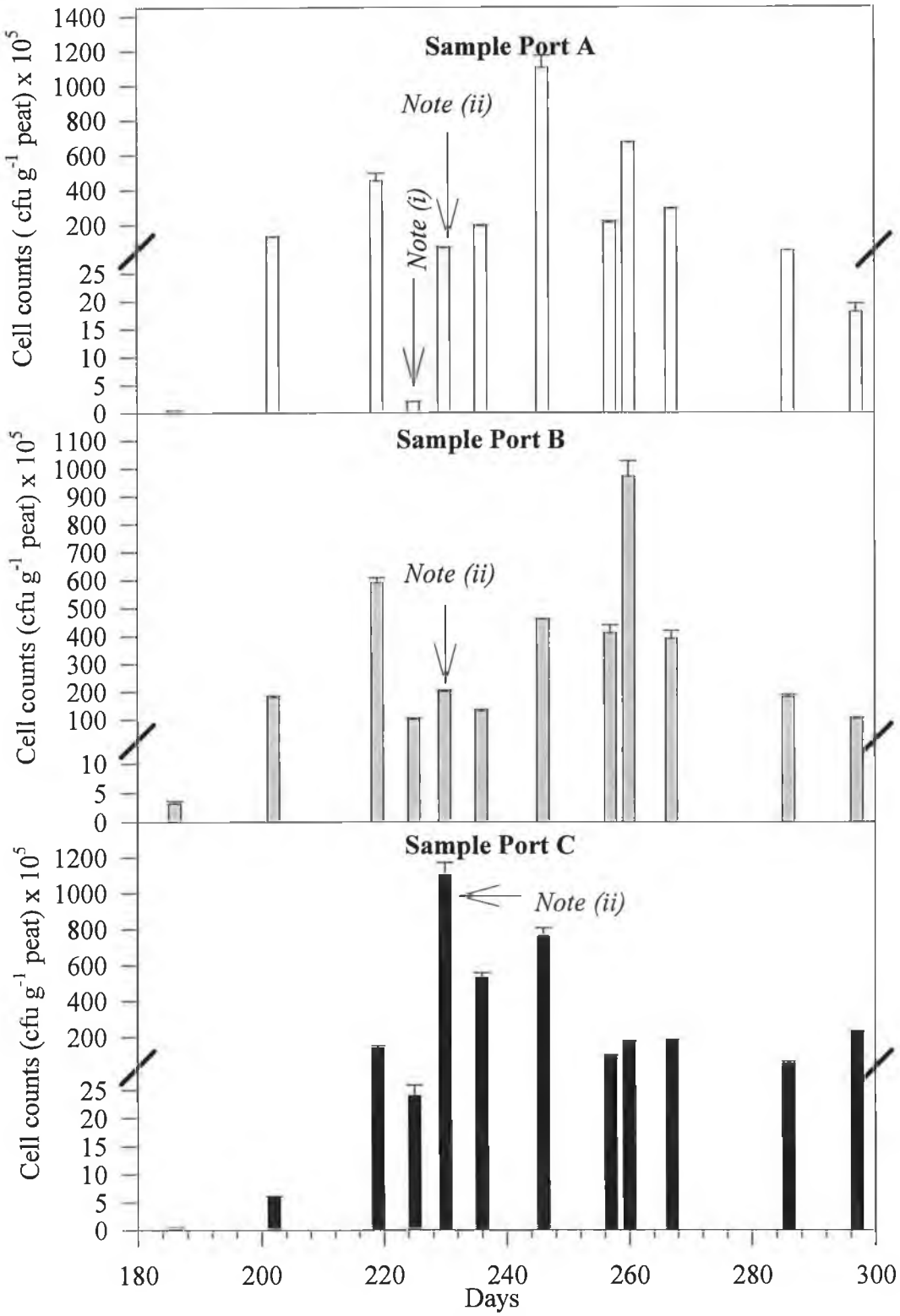
At sample port A, the fungal counts decreased from 6.8 ± 0.2 x 10^3 cfu g^{-1} peat on day 186 to 0 by day 219 when no fungi were detected. The temporary increase in fungal counts between days 225 and 236 was due to the addition of fresh peat to the

biofilter and the inoculation of the peat with activated sludge. By day 246 few fungal cells could be detected (Fig. 24).

Fungal counts at sample port B increased from stage 2 values of $10.0 \pm 0.4 \times 10^3$ cfu g^{-1} peat to $14.0 \pm 1.9 \times 10^3$ cfu g^{-1} peat on day 186. However, despite inoculation of the biofilter with activated sludge containing fungal cells, the numbers of fungi detected at port B were negligible for the remainder of stage 3 (Fig. 24).

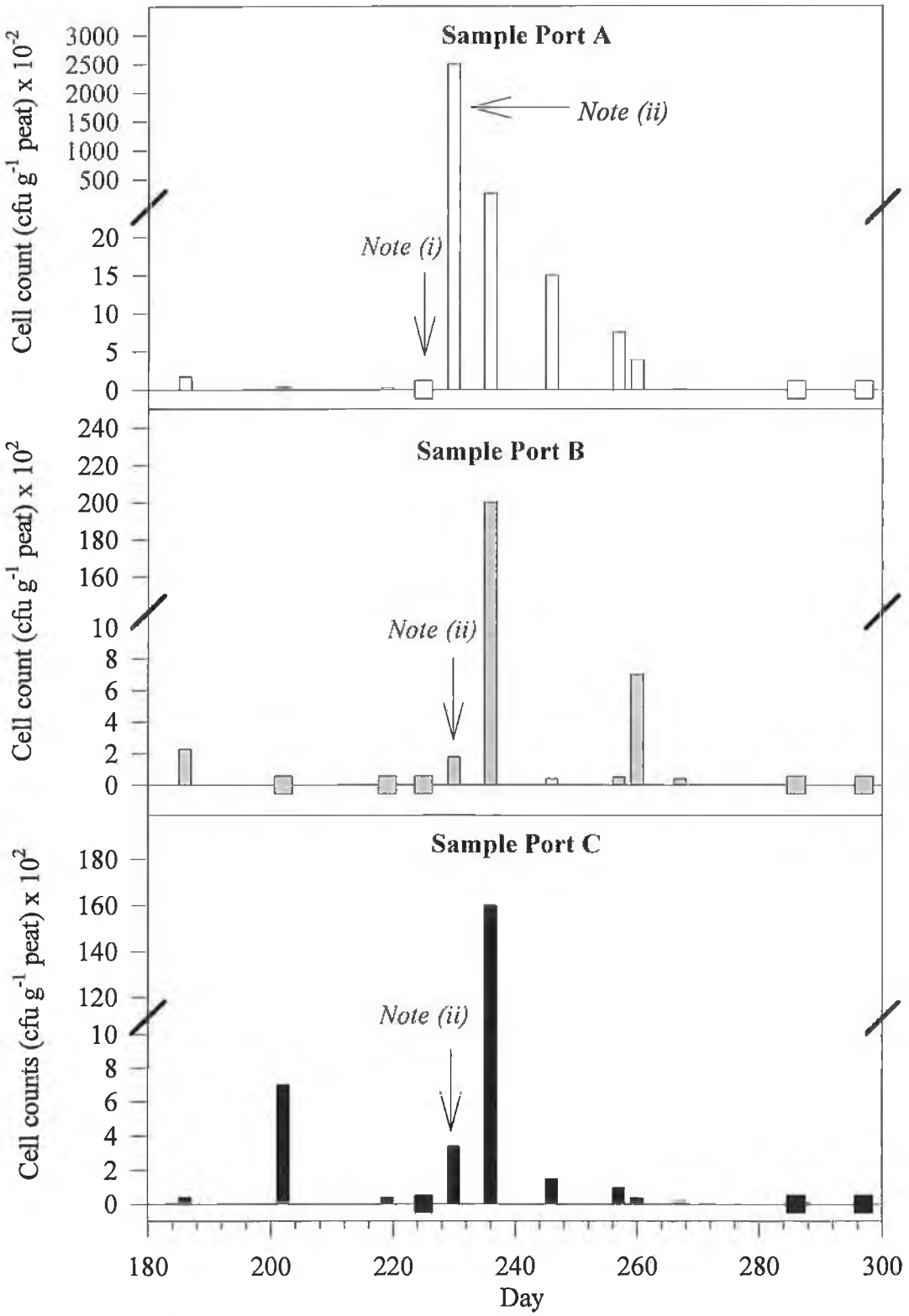
The numbers of fungi detected at sample port C during stage 3 were insignificant. Fungal cells could not be detected even following inoculation of the system with activated sludge. The pH in the biofilter was above pH 7.00 for the majority of stage 3, which would have influenced fungal growth (Fig. 24).

Fig. 22 Bacterial Enumeration of the Peat during Stage 3 of Biofilter Operation



Note (i): Fresh peat added to the biofilter
Note (ii): Counts 24 h after inoculation

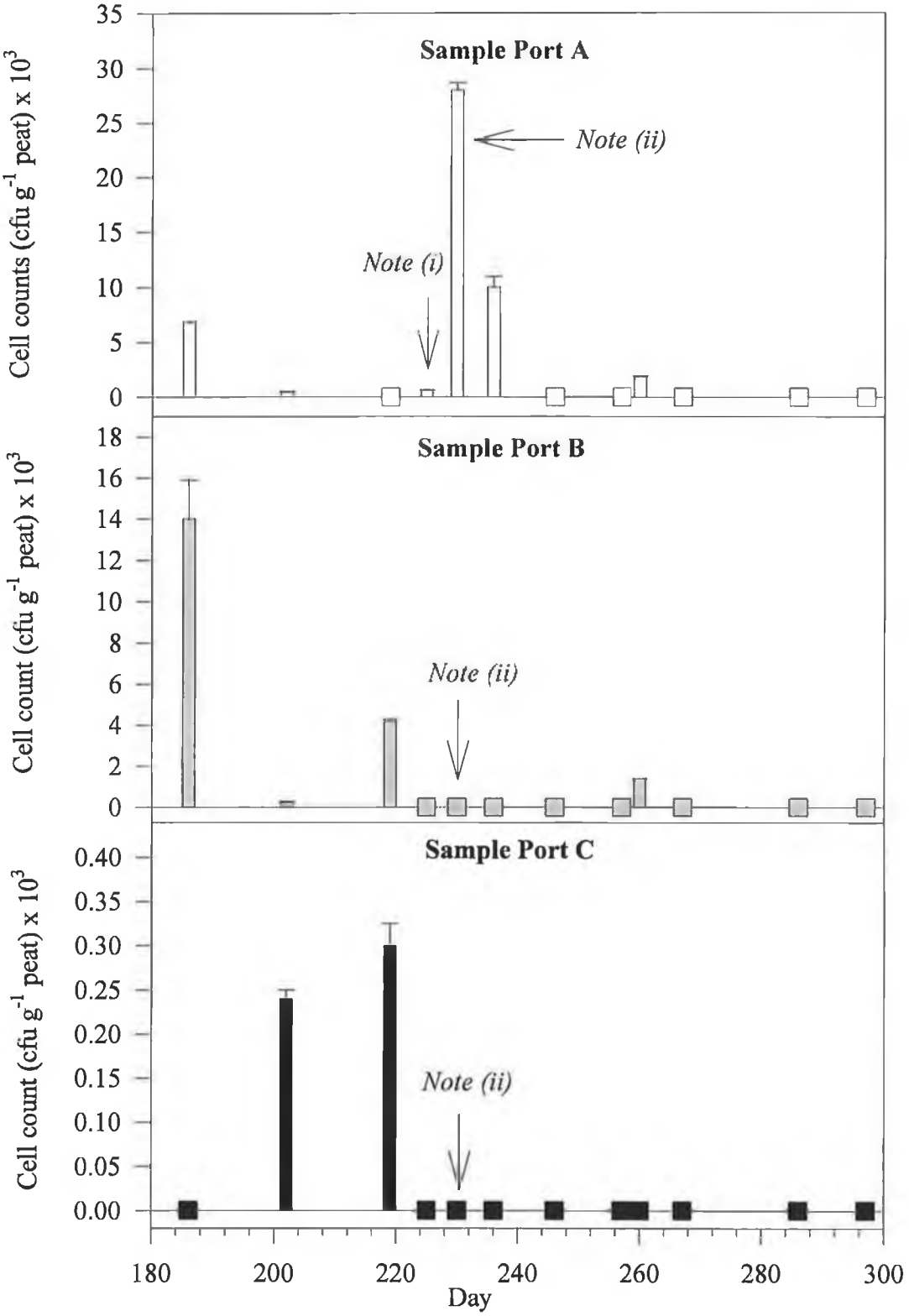
Fig. 23 Nitrifier Enumeration of the Peat during Stage 3 of Biofilter Operation



Note (i): Fresh peat added to the surface of the biofilter

Note (ii): Counts 24 h after inoculation

Fig. 24 Fungal Enumeration of the Peat during Stage 3 of Biofilter Operation



Note (i): Fresh peat added to the surface of the biofilter

Note (ii): Counts 24 h after inoculation

3.5 Biofilter performance overview

The removal of ammonia and the microbiology of the system for all three stages of operation were studied in order to evaluate the overall performance of the biofilter.

3.5.1 Removal efficiency and elimination capacity of the biofilter

The biofilter achieved high removal efficiencies of ammonia from the inlet air stream throughout all three stages of operation. The results are summarised in Fig. 25. During stage 1 there was an acclimation period, during which time the removal efficiency increased to a constant high level (> 90%) which was maintained for stages 2 and 3. However there was a decreasing trend in removal efficiency towards the end of stage 3, indicating that the biofilter was overloaded with ammonia. The consistently high level of removal efficiency in the system showed that the discontinuous mode of operation, the shock loads and shutdown periods had no effect on the removal efficiency of the system.

The high removal efficiency of the system was reflected in the elimination capacity. Figure 26 illustrates how the elimination capacity of the system increased linearly with the mass load for all three stages of biofilter operation. The correlation co-efficient (r^2) was 0.9976.

Fig. 25 Ammonia removal efficiency by peat biofilter seeded with activated sludge

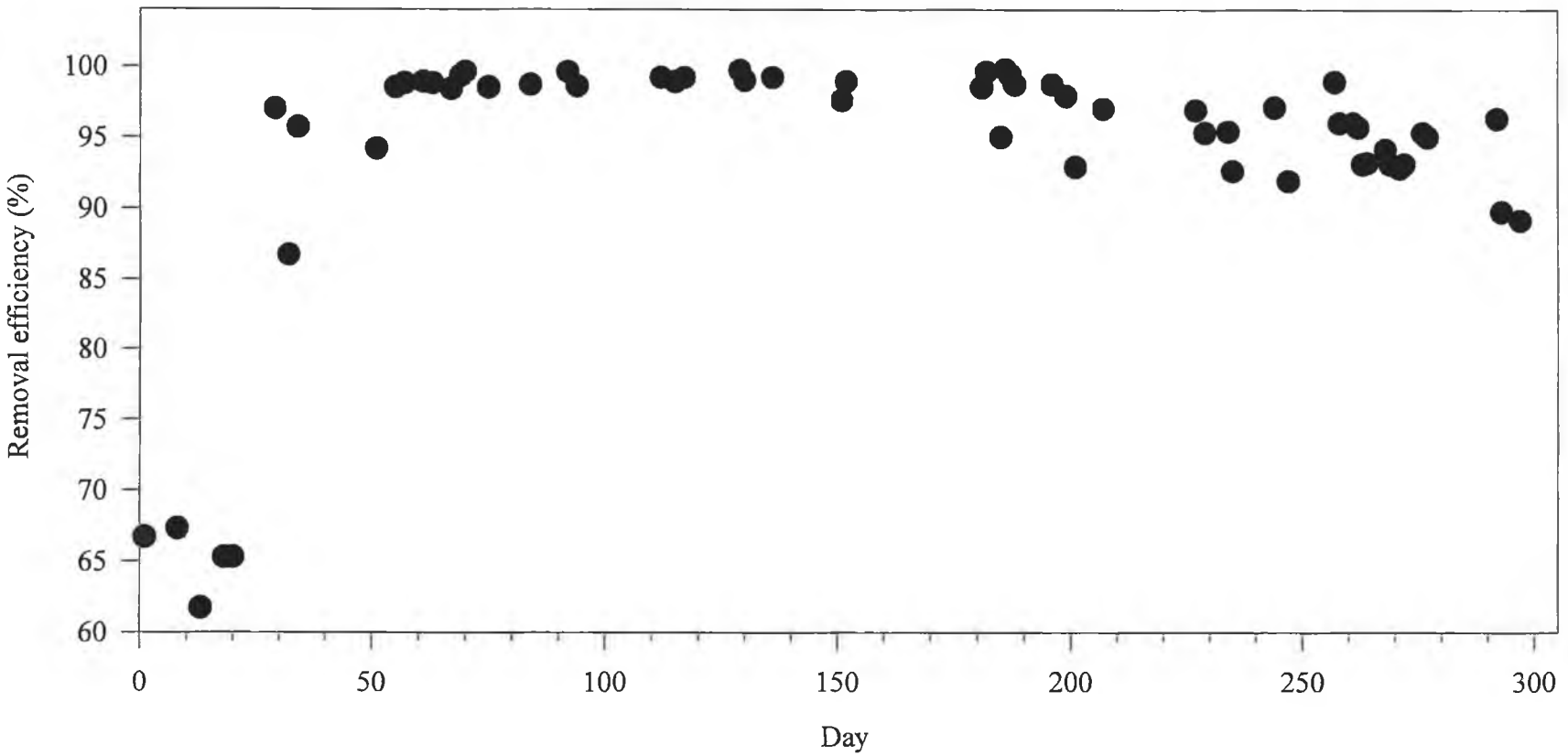
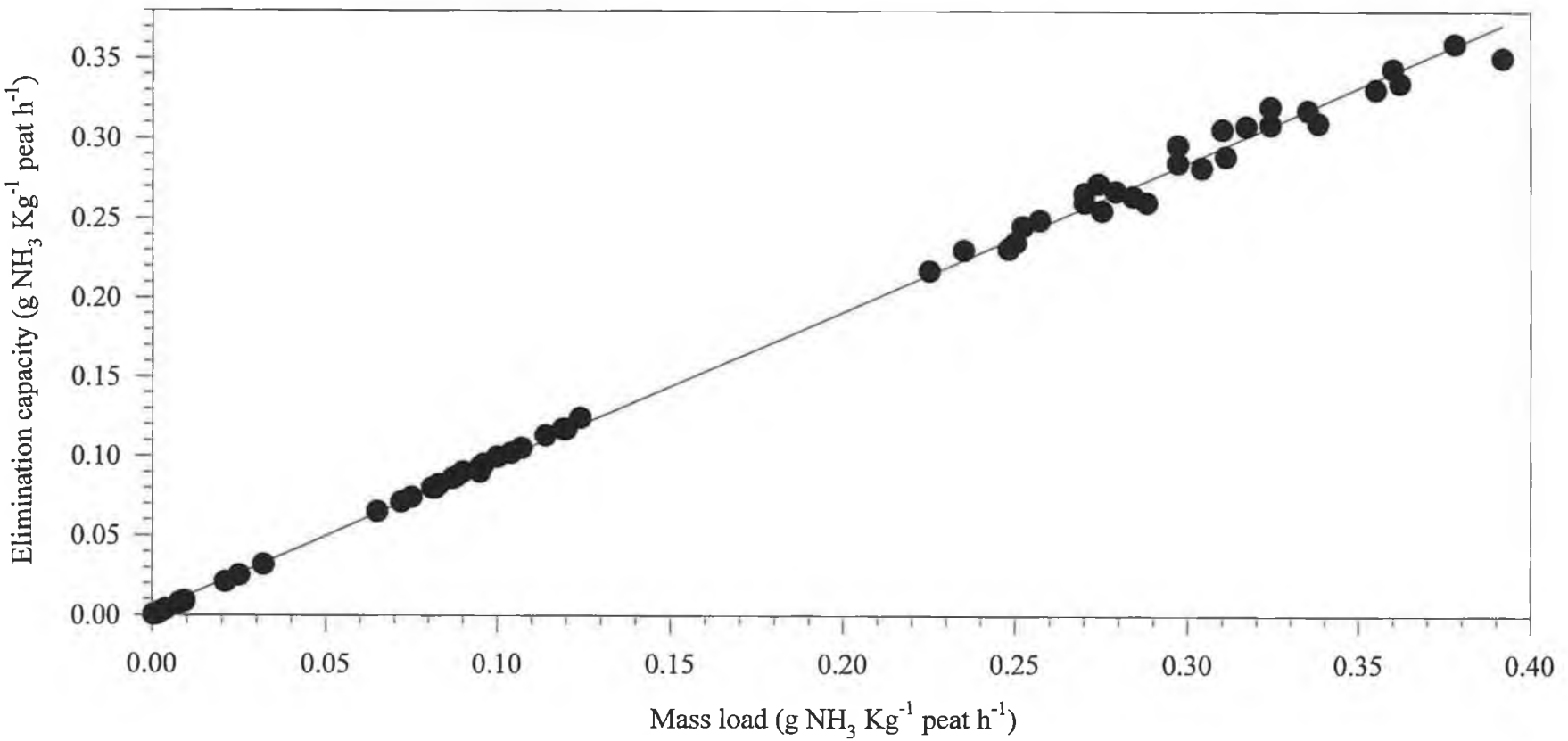


Fig. 26 Relationship between ammonia mass load and elimination capacity of peat biofilter seeded with activated sludge



3.5.2 Overall ammonia balance in the system

In evaluating the overall ammonia balance in the system, the removal of ammonia as described in Sections 3.2, 3.3 and 3.4 together with ammonia lost in the percolate and ammonia adsorbed to the peat was investigated.

Ammonia in / ammonia out

A summary of the results obtained during the three stages of biofilter operation for ammonia in and ammonia out (outlet air and percolate) are described in Table 20.

141.5 mg ammonia was supplied to the filter during stage 1. 14.25 mg emerged in the outlet air and 37.61 mg emerged in the percolate. In total, 51.86 mg ammonia emerged from the biofilter in one or other of these forms, indicating that 37 % of the inlet ammonia was released in untreated form from the system. Of the 49366 mg ammonia supplied to the biofilter during stage 2 only 638.4 mg emerged untreated in the outlet gas stream, as was reflected in the high removal efficiency. 1134.7 mg emerged in the percolate. The ammonia that may have emerged during stage 2 as a result of ammonia supplied in stage 1 was assumed negligible. Therefore the untreated ammonia that emerged from the system during stage 2 was estimated at 3.5 % of the ammonia supplied. During stage 3 very little percolate emerged from the system. However, of the 206530 mg supplied during stage 3, 9359.2 mg emerged in the outlet gas stream and 722.75 mg emerged in the percolate. Overall only 4.9 % of the inlet ammonia was released in untreated form. The value for untreated ammonia in the percolate in stage 3 assumes that the ammonia in the percolate as a result of ammonia supply during stage 2 was negligible.

Table 20 Total amount of ammonia supplied to the filter and total amount of ammonia that emerged untreated

	Ammonia in	**Ammonia out**	
	Inlet air (mg)	**Outlet air (mg)**	**Percolate (mg)**
Stage 1	141.50	14.25	37.61
Stage 2	49366.25	638.43	1134.73
Stage 3	206530.00	9359.20	722.75
Total	256037.75	10012.88	1895.09

Adsorbed ammonia and nitrate

Peat samples taken from the biofilter were analysed for ammonia adsorption at the end of stage 3. The level of ammonia on the native peat was found to be low and ammonia was found to adsorb to the packing material during operation of the biofilter. The amount of ammonia adsorbed by the peat did not vary significantly between samples taken on day 239 and 297 and on day 325, approximately one month following operation of the biofilter. The amount of ammonia adsorbed was also constant along the height of the filter (Table 21). On average the packing material adsorbed 11.2 ± 0.45 mg NH_3 g^{-1} peat, bearing in mind that there was 8 Kg peat in the biofilter, 89600 mg ammonia was found to be adsorbed by the packing material in the latter stages of operation of the biofilter.

Table 21 Ammonia adsorbed by the peat in the biofilter

	Ammonia adsorbed by the peat in the biofilter			
Native peat mg NH_3 g peat^{-1}	**Day**	**Sample port A mg NH_3 g^{-1} peat**	**Sample port B mg NH_3 g^{-1} peat**	**Sample port C mg NH_3 g^{-1} peat**
0.18 ± 0.012	239	9.3 ± 0.8	12.7 ± 1.3	13.5 ± 1.3
	297	12.3 ± 1.0	10.6 ± 0.9	10.1 ±0.9
	325	10.3 ± 1.1	11.1 ±0.9	11.1 ± 1.2

The levels of nitrate present on the peat at the end of stage 3 and on day 325 are described in Table 22. While there was no nitrate detected on the native peat, 1.91 ± 0.12 mg NO_3^- g^{-1} peat was detected on the peat on day 297. One month following the cessation of ammonia gas supply to the biofilter, while the level of ammonia had not decreased (Table 21) the level of nitrate had decreased to 0.33 ± 0.08 mg NO_3^- g^{-1} peat.

Table 22 Nitrate on the peat

	Nitrate on the surface of the peat in the biofilter			
Native peat mg NO_3^- g $peat^{-1}$	**Day**	**Sample port A mg NO_3^- g^{-1} peat**	**Sample port B mg NO_3^- g^{-1} peat**	**Sample port C mg NO_3^- g^{-1} peat**
0	297	2.10 ± 0.12	1.73 ± 0.18	1.77 ± 0.20
	325	0.31 ± 0.02	0.48 ± 0.03	0.19 ± 0.02

Ammonia balance (for all three stages)

- 256037.75 mg ammonia was supplied to the biofilter
- 10012.88 mg emerged in the outlet gas stream
- 1895.09mg emerged in the percolate
- 89600 mg (35%) was adsorbed by the peat
- In total 101471.97 mg ammonia remained untreated
- 39.6 % of the ammonia supplied remained untreated

3.5.3 Microbiology

The average numbers of the various microbial populations investigated for the three stages of biofilter operation are outlined in Table 23. While the overall average numbers of bacteria increased the numbers of both nitrifiers and fungi in the system decreased. The increase in bacterial numbers suggested growth of these microbes at the expense of nutrients in the peat in combination with the gaseous ammonia supplied. As the time of operation of the biofilter progressed, a compaction of the packing material together with deterioration in the integrity of the peat granules was noted. This would have led to an increase in availability of nutrients from the peat for the bacterial population and is reflected in a pronounced increase in bacterial numbers during stage 3.

The native peat was found to have no nitrifiers present (Tables 12 and 16). The numbers of nitrifiers in the system decreased during stage 2 of the biofilter operation. During stage 3, there was a temporary increase in the numbers of nitrifiers following

inoculation of the peat noted at sample port A on days 230 and 236 and at sample ports B and C on day 236. When these counts were excluded from a calculation of the average numbers of organisms, the decrease which occurred in these organisms is more clearly represented (Table 23). The inability of the nitrifying population to establish in the biofilter could have been due to competition from the indigenous bacterial population or the high levels of ammonia adsorbed to the peat, which would have been toxic to the bacteria.

There was a significant decrease in the numbers of fungal cells detected in the system from stage 1 to 2 and again from stage 2 to 3. While fungi were present on the native peat used to pack the biofilter and were also present in the activated sludge inoculum which was added to the biofilter during both stage 1 and stage 3, conditions in the biofilter were not optimal for the growth of fungi. The increase in the water content of the peat, adverse pH (above pH 8) and competition from the bacterial population all prevented optimal growth of fungi in the system.

Table 23: Average microbial counts for the three stages of biofilter operation at each sample port

	Stage 1	Stage 2	Stage 3
Bacteria	CFU g^{-1} peat	CFU g^{-1} peat	CFU g^{-1} peat
Sample port A	$32.5 \pm 11.2 \times 10^4$	$2.5 \pm 0.1 \times 10^4$	$262.0 \pm 96.0 \times 10^5$
Sample port B	$71.5 \pm 26.0 \times 10^4$	$14.9 \pm 2.5 \times 10^4$	$309.3 \pm 78.4 \times 10^5$
Sample port C	$23.6 \pm 4.2 \times 10^4$	$136.4 \pm 67.5 \times 10^4$	$274.0 \pm 99.0 \times 10^5$
Average	$42.5 \pm 14.7 \times 10^4$	$51.2 \pm 42.7 \times 10^4$	$282.0 \pm 14.0 \times 10^5$
Nitrifiers	CFU g^{-1} peat	CFU g^{-1} peat	CFU g^{-1} peat
Sample port A	$2.4 \pm 1.3 \times 10^2$	$0.5 \pm 0.19 \times 10^2$	$^{1}2.9 \pm 1.6 \times 10^2$
Sample port B	$6.6 \pm 2.9 \times 10^2$	$2.2 \pm 0.8 \times 10^2$	$^{2}1.06 \pm 0.7 \times 10^2$
Sample port C	$51 \pm 30.7 \times 10^2$	$34 \pm 28 \times 10^2$	$^{2}1.09 \pm 0.67 \times 10^2$
Average	$20.0 \pm 15.5 \times 10^2$	$12.2 \pm 10.9 \times 10^2$	$1.7 \pm 0.6 \times 10^2$
Fungi	CFU g^{-1} peat	CFU g^{-1} peat	CFU g^{-1} peat
Sample port A	$88 \pm 46 \times 10^3$	$27.9 \pm 7.4 \times 10^3$	$4 \pm 2.4 \times 10^3$
Sample port B	$126.8 \pm 50.5 \times 10^3$	$77.8 \pm 27.6 \times 10^3$	$1.65 \pm 1.2 \times 10^3$
Sample port C	$162.3 \pm 70.1 \times 10^3$	$97.7 \pm 24.7 \times 10^3$	$0.05 \pm 0.03 \times 10^3$
Average	$126.0 \pm 22.0 \times 10^3$	$67.8 \pm 20.8 \times 10^3$	$1.9 \pm 1.1 \times 10^3$

Note:

[1] = day 230 and day 236 (immediately after inoculation) excluded from average.

[2] = day 236 (immediately after inoculation) excluded from average

4.0 DISCUSSION

The biofilter used in this experiment was constructed from perspex with an overall working volume of 0.014 m^3. It was designed based on previously reported successful laboratory – scale biofilters. The working volume was similar to that used by Hartikainen *et al.* (1996), who successfully used the peat biofilter to treat ammonia. The working volume was also similar to the working volumes used by Clark and Wnorowski (1992) who used a compost based biofilter to treat hydrogen sulphide and Tanji *et al.* (1989), who used an immobilised *Thiobacillus* culture on polypropylene pellets to treat sulphur compounds. The physical dimensions of reported peat biofilters used to treat ammonia are outlined in Table 24. All of the biofilters are laboratory-scale with the exception of that used by Martin *et al.* (1996), who operated a pilot-scale system with four biofilters of dimensions as described in Table 24 in series. The biofilter designed and used in this research was within the general size range of other laboratory-scale biofilters.

Table 24 Comparison of physical parameters of peat biofilters successfully used to treat ammonia

Author	Height (m)	Inner diameter (m)	Volume (m^3)
Yani *et al.*(1998)	0.5 (biofilter) 0.14 (packed)	0.05	0.0098 (total) 0.000275 (packed)
Hartikainen *et al.* (1996)	0.9 (biofilter) 0.45 (packed)	0.2	0.028 (total) 0.014 (packed)
Martin *et al.* (1996)	0.6 (biofilter) 0.4 – 0.5 (packed)	0.45	0.095 (total) 0.064 – 0.08 (packed)
Togashi et al. (1986)	0.5 (biofilter)	0.15	0.0088 (total)
Biofilter used in this research	0.6 (biofilter) 0.45 (packed)	0.2	0.0188 (total) 0.014 (packed)

Perspex was chosen as the material of construction for the biofilter used in this investigation because it is a transparent material. One of the advantages associated with the use of transparent materials for construction is that moisture content of the packing material and holes that develop in the bed can be observed. Perspex is a high tensile strength material, it is resistant to both acids and alkalis and it is impermeable to gases (http://www.bibby-sterilin.com/cat/azlon/acrylic.htm and http://www.plasticsusa.com/pmma.html). Almost any kind of material can be used for the physical construction of biofilters. Most reported laboratory-scale biofilters, which were used to treat a variety of pollutants using both organic and inorganic packing materials, were constructed from transparent materials including glass (Barnes *et al.*, 1995, Chung and Huang, 1998, Marek. *et al.*, 1999, Yani *et al.,* 1998 and Zilli *et al.*, 2000) and/or plastic and synthetic derivatives including PVC (Bibeau *et al.*, 1997, Hartikainen, *et al.*, 1996, Hirai *et al.*, 1990 and Togashi *et al.*, 1986), plexiglas (Smet, *et al.*, 1996 and Weckhuysen. *et al.*, 1994), perspex (acrylic) (Brennan *et al.*, 1996, Clark and Wnorowski, 1992, Deshusses *et al.*, 1996, Elsgaard, 2000, Quinlan *et al.,* 1999 and Yang and Allen, 1994), and plastic (Degorce-Dumas *et al.*, (1997). Traditionally full-scale closed systems were constructed from concrete or as steel frames with sheet metal and they were installed at ground level. In recent years plastic systems have become more popular because space constraints led to the development of roof-top installations and roof-tops can not support the concrete systems Swanson and Loehr (1997). Cho *et al.* (1991) constructed a pilot-scale biofilter from steel.

The biofilter used in this research was a closed system and was operated in an upflow manner based on the design used by Hartikainen *et al.* (1996). According to Allen Boyette (1998), the majority of operating biofilters in the United States are constructed as open structures because they are cheaper to build and they achieve similar removal efficiencies as closed systems. However, research systems tend to be closed structures as it is easier to monitor control parameters e.g. moisture in closed systems. Upflow and downflow biofiltration units are used with equal frequency. Hartikainen *et al.* (1996) and Martin *et al.* (1996) both used up-flow systems and M^{c}Nevin *et al.* (1999) and Yani *et al.* (1998) used down-flow systems to treat ammonia in peat biofilters. Krailas *et al.* (2000) treated methanol emissions using a compost-based biofilter in both an upflow and a downflow mode. Both methods of operation resulted in an elimination capacity of 101 g MeOH m^{-3} packing h^{-1} when

the mass load was 169 g MeOH m^{-3} packing h^{-1}. Most simple full-scale biofilters are shallow pits with the air input pipe installed at the bottom and are therefore up-flow systems (Devinny, 1998). However down-flow operation prevents drying out of the lower parts (the sprinkler is at the top) and limits the discharge of VOCs dissolved in the drainage water (van Groenestijn and Hesselink, 1993).

A sieve plate was also installed in the biofilter used in this study to ensure even distribution of incoming air through the bed and to prevent by-passing around the edges (Wani *et al.*, 1997). Other authors who reported the use of sieve plates to ensure homogeneous dispersion of the gas through the packed beds included Chung *et al.* (2000), Hartikainen *et al.* (1996) and van Langenhove *et al.* (1988).

Peat was chosen as the packing material for this research because it has been widely and successfully used in biofiltration. Hartikainen *et al.* (1996), Martin *et al.* (1996), M^{c}Nevin *et al.* (1999), Togashi *et al.* (1986) and Yani *et al.* (1998) all used peat beds to treat ammonia in laboratory-scale and pilot-scale systems. It has good absorption/adsorption properties, high cellulose content, large moisture retention capacity, good buffering capacity and is widely available (Wani *et al.,* 1997). Peat has also been used as the carrier material in biofilters that treated toluene (Acuna *et al.*, 1998 and Bibeau *et al.*, 1997), dimethyl disulphide (Cho *et al.*, 1991), methylamine (Chou and Shiu, 1997) and sulphur based odours (Brennan *et al.*, 1996). Also Oh and Choi (2000) compared the effectiveness of different organic packing materials, including peat in a biofilter used to treat toluene and m- and p-xylene. They got the greatest removal efficiency of all three substrates when they used peat as the packing material biofilter compared with other organic materials such as bark chips, vermiculite and hydroballs. The peat biofilter achieved more than 82 % removal efficiency for all three substances whereas the removal efficiencies achieved with the other materials varied from 10.1 % to 58.6 %.

However, there is a huge range of materials, both organic and inorganic, that can potentially be used as the packing material for biofilters. Theoretically biodegradation can occur on any media once it is biologically active (Wani *et al.*, 1997). Kim *et al.* (2000) found that organic packing materials achieved higher removal capacities of ammonia (when the removal capacity was calculated based on the bed volume) than inorganic materials when operated at inlet concentrations of 0 – 300 ppm (0 – 228 mg m^{-3}). On a volume basis the complete removal capacity for the peat, rock wool, fuyolite and ceramics was 1.0 x 10^3 g N m^3 d^{-1} (50 g NH_3 m^{-3}

packing h^{-1}), 1.2 x10^3 g N m^3 d^{-1} (60.7 g NH_3 m^{-3} packing h^{-1}), 6.8 x 10^2 g N m^3 d^{-1} (34.4 g NH_3 m^{-3} packing h^{-1}), 9.2 x 10^2 g N m^3 d^{-1} (46.5 g NH_3 m^{-3} packing h^{-1}) respectively. The removal capacity can also be calculated on the basis of bed weight, in which case the fuyolite had the highest capacity at 4.7 g N Kg^{-1} dry material d^{-1} (0.24 g NH_3 Kg^{-1} packing h^{-1}) and the ceramics had the lowest at 2.4 g N Kg^{-1} dry material d^{-1} (0.12 g NH_3 kg^{-1} dry material d^{-1}). Differences in the elimination capacity based on bed volume and bed weight were due to differences in packed densities. The use of organic materials is preferable because from the point of view of engineering the compactness of the filter is of primary concern and organic packing materials provide for more compact beds.

Sometimes bulking agent, such as glass beads are mixed with the organic materials, such as peat to minimise compaction. The presence of glass beads was also found to aid distribution of the inlet gas stream throughout the bed (Zilli *et al.*, 1996). Included in the organic and inorganic packing materials that have also been used in the biofiltration of ammonia are wood bark (van Langenhove *et al.*, 1988) and Fuyolite (Kim, *et al.*, 2000).

The peat was sieved, neutralised and inoculated with activated sludge prior to use in the biofilter. The peat was sieved prior to packing because it is recommended that the d_{60} of the packing material be greater than 4 mm (i.e. 60 % of the particles must have a diameter of 4 mm or more) to prevent pore clogging by smaller particles and fines (Leson and Winer, 1991).

Nitrifiers enjoy a neutral environment and in order for the carrier peat to support the growth of such organisms it was neutralised prior to packing. Hartikainen *et al.* (1996), Togashi *et al.* (1986) and Yani *et al.* (1998) all neutralised peat prior to packing the filters. It was found that when the peat was seeded with activated sludge, removal efficiency was higher if the peat was neutralised prior to inoculation (Togashi *et al.*, 1986). Martin *et al.* (1996) used peat that had an initial pH of pH 4.0, which rapidly increased to pH 8.0 upon the supply of ammonia to the system. Neutralisation of peat minimises adsorption to the packing and therefore ensures removal by biological means.

The advantage of inoculating biofilters has been widely argued. As many packing materials are of natural origin and therefore have a native population of microbes present the usefulness of inoculating such materials has been questioned (Wani *et al.* 1997). It is argued that shorter adaptation times can be obtained by inoculation with

specialised microorganisms (van Groenestijn and Hesselink, 1993). However, it is also argued that although microbial infusions, or seeding, might reduce the adaptation time in some cases it does not seem to be very practical because large populations of native microbes swamp out and rapidly consume invading inoculants. A better approach is to pre-treat the bed with pulses of the VOC before placing the biofilter onstream. Upon the supply of a specific substrate (target pollutant) it is expected that the distribution of the microbial population would shift towards strains that can metabolise the target pollutant (Leson and Winer, 1991) and therefore inoculation should not be necessary in organic materials. In the long run, biofilters rely on the ability of the native microbial population to adapt to the VOC (Bohn 1993). Smet *et al.* (1999) found no difference in the elimination capacities of a fresh compost biofilter used to remove ammonia from a waste gas stream before and after inoculation. Elimination capacities of up to 350 g NH_3 m^{-3} d^{-1} (14.6 g NH_3 m^{-3} h^{-1}) were obtained in both the inoculated and non-inoculated biofilter. The cumulative ammonia removal over 73 days of operation was 9.3 g NH_3 Kg^{-1} compost when the biofilter was inoculated. Although the same removal capacity was achieved when the biofilter was not inoculated, it only lasted 18 d, after which there was a significant fall off in elimination capacity. Complete breakthrough occurred by day 27 of operation. However the cumulative amount of ammonia removed was 7.9 g NH_3 Kg^{-1} compost in the non-inoculated biofilter, which was very similar to the amount removed by the biofilter that was inoculated. They concluded that inoculating the compost had no effect on the ability of the filter to remove ammonia. Although complete breakthrough occurred much quicker when the filter was not inoculated it was supplied with a higher load of ammonia. Apparently the compost used was a good inoculum for nitrifying micro-organisms.

Although the usefulness of inoculation is debated it has become common practice to inoculate biofilters because it does reduce the adaptation time (Wani *et al.* 1997). As the peat used in this study had no nitrifiers in the native microbial flora and Hartikainen *et al.* (1996) found that native peat had no nitrification capacity it was deemed necessary to inoculate the peat with nitrifiers to ensure biological removal of ammonia in the biofilter. Togashi *et al.* (1986) also examined the removal of ammonia from a waste gas stream in a peat biofilter before and after the peat was inoculated with nitrifying activated sludge. When the filter was not inoculated with nitrifying activated sludge breakthrough occurred within 20 days depending on the

loading indicating that the initial ammonia removal was due to adsorption of ammonia to the packing i.e. accumulation within the filter. Complete removal of 40 ppm (30.4 mg m^{-3}) ammonia at a mass load of 0.16 g N Kg^{-1} dry peat d^{-1} (0.008 g NH_3 Kg^{-1} peat h^{-1}) was achieved for 101 days of operation when inoculated peat was used in the biofilter. Nitrites and nitrates were produced indicating that removal was due to biological activity.

Activated sludge was chosen over the pure culture as the inoculum because laboratory grown microorganisms tend to be much more sensitive than free-living organisms to environmental conditions (Boch *et al.*, 1991). And based on the fact that the peat used had a substantial native microbial population, it was thought that the pure cultures would be rapidly consumed under biofilter conditions.

Mixed microbial populations are the preferred inoculum for biofilters that employ organic packing materials nevertheless pure cultures have been used to successfully treat ammonia in inorganic beds. Kim *et al.* (2000) seeded fuyolite (a type of perlite) with a marine bacterium *Vibrio alginolyticus* and Chung and Huang (1998) immobilised *Nitrosomonas europaea* in calcium-alginate beads. Kim *et al.* (2000) supplied ammonia at concentrations varying from 200 μL L^{-1} (152 mg m^{-3}) to 1200 μL L^{-1} (911 mg m^{-3}) over 61 days of operation and achieved over 85 % efficiency. Chung and Huang (1998) achieved 97.5 % removal efficiency when ammonia was supplied at concentrations less than 100 ppm (75 mg m^{-3}).

One of the most important parameters of the packing material is the moisture content and it is also the most difficult parameters to control. Insufficient moisture in the media caused a reduction in biofilm thickness. According to Gostomski. *et al.* (1997) and Standefer and Willingham (2000) it is more important to maintain uniform moisture throughout the media rather than targeting the 'optimal' moisture content, which is not well established. However, Leson and Winer (1991) and Utkin *et al.* (1990) recommended that peat based packing materials should be maintained at a moisture content of 40 – 60 %, which was the moisture content targeted in this research. The fact that many full-scale biofilters are open systems suggests that the moisture of the system is somewhat excluded from the design even though sprinklers are generally installed. Sometimes biofilters are mounted on load cells where decreases in weight indicates that the moisture content has decreased and the filter bed is then automatically sprinkled (Devinny, 1998). Moisture levels in all the

reported laboratory scale biofilters for treating ammonia were controlled by adding water to the surface of the bed, it was also the method used in this research. During stage 3 it became apparent that the method was not sufficient as cracks developed in the peat bed, which caused channelling and thus emergence of untreated ammonia from the biofilter. Cardenas-Gonzalez *et al.* (1999) evaluated the media of a full-scale biofilter used to treat VOC's. The media in the filter was compost and among the parameters monitored was moisture content. During the first three years of operation there was no moisture control and it was an open system. The moisture content varied from 16 % to 71 %. A sprinkler system was employed after three years and although the moisture content improved it was still highly variable at 24 % - 80 % indicating that addition of water to the surface of the biofilter was not the most appropriate method of maintaining moisture. The increase in moisture resulted in better pH control. They also found that both high (76 - 80 %) and low (24 %) moisture contents inhibited aerobic microbial activity. However it is assumed that the removal efficiency of the biofilter was adequate throughout the five years of operation and that the large variation in moisture content did not affect the overall ability of the biofilter to remove VOC's. A large number of samples were required to represent the entire bed due to the heterogeneity of the material. However the number of samples taken from the bed was minimised as removal of samples lead to the development of holes and resulted in channelling of the air (Cardenas-Gonzalez *et al.*, 1999). For this reason and the fact that irreversibly trapped ammonia on the surface of the peat was also released during oven drying the number of peat samples taken from the filter bed for moisture analysis in this investigation was minimal and moisture control was based on visual inspection. The moisture content is only an indirect indicator of what is available to the resident microbes (van Lith *et al.*, 1997). All other authors pre-humidified the inlet gas stream in order to aid maintenance of the moisture level throughout the system. According to Wani *et al.* (1997) a relative humidity of 95 % in the inlet air is sufficient but moisture will be continually stripped from the bed if the inlet air is not saturated to greater than 99 % relative humidity. Although the air entering the biofilter in this research had a much lower relative humidity than is recommended (45 – 70 %) it was compensated for by the sprinkler system. The outlet air, as already stated, emerged at a higher relative humidity and higher temperature than the inlet gas. The increase in the gas temperature may be due to heat exchange with the environment (external conditions

– air surrounding the biofilter) or may be due to exothermic reactions such as biooxidation within the biofilter. As the temperature of the air increases it causes moisture to evaporate and therefore the air becomes more saturated and the relative humidity increases (van Lith *et al.*, 1997).

Waste gases from intensive farming practices contain a multitude of components, many of which combined, lead to the odour associated with the industry. Ammonia is a known constituent of such gas streams and was therefore chosen as a model substrate because the biofilter was a laboratory system and it was therefore impossible to supply it with the mixture of gases responsible for farming odours. Ammonia is widely documented as a pollutant from other industrial sources also (Section 1.1.2). It is also well suited to biofiltration as it is a biologically degradable compound.

Discontinuously operated biofilters is not a widely studied phenomenon but Leson and Winer (1991) have suggested that most industrial sources of air pollutants do not operate continuously. It is therefore of interest whether the biological activity of the biofilter would suffer due to such discontinuous supply of substrate to the micro-organisms present. Ottengraf and van den Oever (1983) suggested that microbes could survive up to 2 weeks without any significant loss in activity in a peat compost biofilter used to treat an inorganic gas stream that contained toluene.

The method of trapping ammonia was a modification of the method documented by Harrison (1986), who recommended that gaseous ammonia be sampled by bubbling it through weak acid, (0.025 M H_2SO_4) for 30 min. at a rate of 30 L min^{-1}. The concentrations of ammonia supplied to the filter saturated the acid within 30 minutes therefore shorter sampling times were examined. Hartikainen *et al.* (1996) aspirated a known volume of gas through three standard impingers containing 0.01 N H_2SO_4 to trap the ammonia. For the current study it was found that a stronger acid (0.05 M H_2SO_4) and four gas-washing bottles in series were required to trap all the ammonia from each sample of inlet air taken. Three minutes sampling time was sufficient. Two colorimetric methods, the Nessler and indophenol blue method and the ammonia ion specific electrode were examined as potential methods for ammonia concentration determination in this research.

The colorimetric methods were found to be unsatisfactory because

1. the samples needed to have an ammonia concentration below 10 mg L^{-1} to be within the linear range for Nesslerisation, (Greenberg *et al.*, 1992) and the indophenol blue method yields reproducible results in the range 0.02 – 1 mg ammonia L^{-1} only, (Harrison 1986), both of which are well below the concentration ranges of the samples used in this research.
2. both methods use toxic reagents, Nessler reagent in the case of the Nessler method and phenol-nitroprusside in the case of the indophenol method, which have to be subsequently disposed of.
3. both methods require a 30 minute development time
4. compounds such as sulphides that may be present in ambient air interfere with the Nessler method.

The ammonia ion specific electrode for the determination of gaseous ammonia concentrations was also used by Hartikainen *et al.* (1996). Other authors have successfully used alternative methods for gaseous ammonia concentration determination including the colorimetric Nessler method, that was found unsuitable in this experiment (Martin *et al.*, 1996) and Gastec detector tubes (Togashi *et al.*, 1986). Chung and Huang (1998) used both Gastec detector tubes and a Single Point Monitor for continuous measurement of the ammonia concentration.

The initial flowrate of gas supplied to the biofilter in this investigation was 23 L min^{-1}, which corresponded to the 37 s residence time was based on flowrates used by Hartikainen *et al.* (1996). They operated their biofilter at two different flowrates. Initially a flowrate of 2.4 m^3 h^{-1} (40 L min^{-1}), which corresponded to an empty bed contact time of 21 s, was used and ammonia was supplied at a concentration of 0 – 47 mg m^{-3}. Ammonia removal efficiency was low after two weeks of operation. As a result of inefficient ammonia removal the inlet concentration was reduced to 14 mg m^{-3} and the flowrate was reduced to 1.8 m^3 h^{-1} (30 L min^{-1}), which corresponded to an empty bed contact time of 28 s. Ammonia was successfully removed for the 48 d of operation under those operating conditions. In this research the starting flowrate was chosen to be lower (and thus a longer empty bed contact time) than that used by Hartikainen *et al.* (1996), when they successfully treated ammonia because the inlet ammonia concentration was slightly higher than that used by Hartikainen *et al.* (1996).

At the flowrates used during stage 1 there was pressure build-up in the humidifier and caused it to leak. Although the volume of the packed beds used in this research and used by Hartikainen *et al.* (1996) were equal, the overall height of the biofilter used by Hartikainen *et al.* (1996) was 0.9 m, which meant that they operated with a larger headspace and therefore encountered no pressure problems at high flowrates. As a result of the pressure build-up the flowrates were reduced during stage 2. Although, with a flowrate of 18 L min^{-1} the EBCT was 47 s which was high compared to the EBCTs reported by Hartikainen *et al.* (1996) and Martin *et al.* (1996) it was found to be the optimal flowrate in that pressure build-up problems were eliminated and also the EBCT was sufficient to remove ammonia from the inlet gas stream. Martin *et al,* (1996), who supplied ammonia at an inlet concentration of 20 – 30 mg m^{-3} reported the shortest empty bed contact time of 7 – 25 s for ammonia removal using a peat biofilter. However they used a pilot scale system with 4 columns in series, each one with a packed volume of 0.064 – 0.08 m^3. The peat used in the column was not neutralised prior to packing therefore the adsorptive capacity of the peat was higher than reported values as it was common practice to neutralise the peat. Also the volume of material available to adsorb the ammonia was a lot greater than the volume available in the biofilter used in this research. Smet *et al.* (2000) found that when ammonia was supplied to a compost biofilter inoculated with nitrifying culture at inlet concentrations of 190 – 310 mg m^{-3} that an EBCT of 40 s was not sufficient to effectively treat the incoming load. Efficiency was 64%. Although at the same inlet ammonia concentrations 94 % removal was achieved with an EBCT of 131 s.

The removal efficiency is a direct measure of the efficiency of a biofilter to remove a pollutant from a waste gas stream. In determining the removal efficiency only pollutant that emerges in the outlet air stream is accounted for, therefore in order to determine the usefulness of biofiltration as an air pollution control mechanism a clearer knowledge of the fate of the pollutant is desirable. For this reason untreated ammonia that emerged in the percolate was examined and the microbiology of the biofilter was monitored throughout operation.

The percolate was used as an indicator of changes in the biofilter environment. The volume of percolate that arises is a function of the water added to the biofilter for moisture maintenance. Ideally there should be no percolate from a biofilter but in the

case of a research system, percolate is necessary to monitor changes in the peat bed reactor. Any excess water added to the system used in this research percolated through the peat bed and did not lead to over-watering until the bed began to compact. As ammonia concentration was measured using the ion selective electrode, the ammonia was required to be in the liquid phase. Continual measurements within the filter bed were impossible. Likewise nitrate in the system could only be measured from the liquid phase as continual removal of peat samples would have lead to the development of holes in the peat bed, which in turn would have lead to a decrease in efficiency. The percolate is the quickest and most convenient method for detecting changes in the biofilter. It was the method employed by Hartikainen *et al.* (1996) to determine nitrite and nitrate concentrations in the filter. Yani *et al.* (1998) also used the percolate to determine the presence of nitrites and nitrates. Also Hartikainen *et al.* (1996) extended pH electrodes along the height of the filter and found the pH of the percolate was similar to the pH determinations using the electrodes, which indicates that the percolate is an accurate measurement of the situation within the filter.

Changes in the microbial community were monitored in order to gain a clearer understanding of the processes involved in the elimination of ammonia from a waste gas stream. Fungal and bacterial populations were enumerated because they are known to be present on peat from previous experiments performed in the laboratory and changes in either populations are indicative of environmental changes within the peat bed. The nitrifier population was examined because it is the most important microbial group in terms of biological ammonia removal.

The most probable number method for determining nitrifier numbers was performed using a micro and a macro technique. Both methods yielded the same results and the macro technique was used for the majority of the analyses because it was easier to use.

Peat is a very heterogeneous material and variations in cell numbers may be attributed to sampling difficulties. All the samples were taken from the surface and the edges of the peat bed. Obtaining samples in the middle of the bed was impossible and it would have lead to the disruption of the established biofilm. Nitrifying organisms are light sensitive and therefore the numbers at the surface of the bed may have decreased while populations within the bed may have thrived and remained undetected throughout the period of high removal efficiency.

The plate count technique is a convenient method for enumerating the overall microbial population, however it only takes into account culturable micro-organisms, which are generally representative of only a small number of the micro-organisms present. No one medium nor any one temperature will support the growth of all possible organisms Similarly each colony develops from a viable unit, which, may be as a result of a single cell or thousands of cells thus making reproducibility difficult. Cells are seldom evenly distributed throughout a sample and therefore large errors are incurred (Collins and Lyne 1984).

During stage 1, when the average inlet concentration of ammonia was 13.9 mg m^{-3} (mass load = 0.0023 g NH_3 Kg^{-1} peat h^{-1}), the initial removal efficiency was 61.7 – 67.3 %, after which the efficiency increased and remained high for the rest of the experiment. An acclimation period, as was observed in this investigation, is expected when mixed microbial populations are used to inoculate the filter. According to Utkin *et al.* (1990) once a biofilter has started to operate, the efficiency of the system will gradually increase until it reaches a definite level as the micro-organisms adapt to growth on the new substrate. The adaptation period depends on the nature of the compounds involved and the microbial population, and may last from several hours to several weeks. However the acclimation period in this research was not accompanied by an increase in the microbial populations as is expected. The fungi closest to the ammonia inlet were the only group that increased after the period of acclimation but fungi are considered to be the most efficient and abundant of the heterotrophic nitrifiers (Kuenen, 1988). In a study involving the examination of nitrification inhibitors, conducted by Anthonisen *et al.* (1976), it was hypothesised that any lag experienced before nitrification was due to inhibition by free ammonia rather than an acclimation period because all units were inoculated with mixed liquor from a completely nitrifying unit. As the biofilter in this research was inoculated with nitrifying sludge, there is a distinct possibility that the period of low removal was influenced by inhibition within the system because the inoculated microbial population were already accustomed to ammonia substrate.

In this research it was also expected that the period of acclimation would not be evident, as complete removal should be achieved due to the physico-chemical properties of peat. Peat has been found to possess unique chemical and physical properties because lignin and cellulose, major components of the peat, bear polar

functional groups including acids, alcohols, aldehydes, ethers, ketones and phenolic hydroxides, all of which may be involved in chemical bonding (Martin, 1991). However, the adsorptive capacity of the peat was minimised by neutralisation with $Ca(OH)_2$. Hartikainen *et al.* (1996) found that untreated peat was capable of adsorbing 17.9 g NH_3 Kg^{-1} peat whereas neutralised peat only adsorbed 8.6 g NH_3 kg^{-1} peat. Nevertheless Yani *et al.* (1998) observed no microbial start-up period even though they neutralised their packing material. They achieved complete removal of ammonia during the first 20 days of operation due to physico-chemical interactions of ammonia with the peat.

Overall the adsorptive capacity of the peat used in this research was 11.2 ± 0.45 mg NH_3 g^{-1} peat and it was considered that most of the adsorption should have occurred during the early stages of operation, which clearly was not the case. The overall adsorptive capacity was similar to adsorptive capacities of neutralised peat examined by Hartikainen *et al.* (1996), who experienced no acclimation period after biofiltration start-up. Togashi *et al.*, (1986) reported that the adsorptive capacities of various peats ranged from 10 – 20 g N Kg^{-1} peat (12 – 24 g NH_3 Kg^{-1} peat), which was greatly reduced by neutralisation. McNevin *et al.* (1998) also examined the adsorptive capacities of peat and found that oven dried samples adsorbed 0.14 ± 0.04 g NH_3 g^{-1} peat (140 g NH_3 Kg^{-1} peat), which was significantly higher than previously reported values and values found during this research. Moist samples of peat were found to adsorb 0.45 ± 0.08 g NH_3 g^{-1} peat (450 g NH_3 Kg^{-1} peat), indicating that the moisture content influences the adsorptive capacity of the peat. The moisture content of the peat in the biofilter used for this research probably had a significant role to play in the lack of adsorption during the very early stages of operation, although it was maintained at the recommended 40 – 60 %.

The low efficiency achieved in the early part of this research may however have been due to gas-channelling along the walls of the column, before the peat bed settled or it may have been due to the shorter empty bed contact time of 37 s that was used during stage 1 compared to stages 2 and 3. Although the removal efficiency during the early part of stage 1 was lower than anticipated there was still at least 65 % removal of ammonia from the inlet gas stream in each run. There was evidence of ammonia removal through accumulation in the biofilter and through nitrification as indicated by the presence of ammonia and nitrate in the percolate. During the period

of low removal ammonia accumulated at a faster rate than nitrate was produced as suggested by the increase in percolate pH. Martin *et al.* (1996) reported that the pH of their peat increased from pH 4.0 to approximately pH 8.0 during the first 10 - 20 days of operation when ammonia was supplied at an inlet concentration of 20 – 30 mg m^{-3}. They extracted 12 mg N g^{-1} peat (14.7 mg NH_3 g^{-1} peat) from the peat during that period, which is similar to previously reported adsorptive capacities of peat. In this research nitrification by autotrophic organisms was supported by a temporary increase in nitrifiers at sample port C, closest to the ammonia inlet upon the supply of ammonia. The nitrifier numbers increased from the initial value of 40 x 10^2 cfu g^{-1} peat to 140 x 10^2 cfu g^{-1} peat on day 7.

The biofilter had acclimated to stage 1 ammonia supplies by day 29, nevertheless much of the removal was due to absorbency of the ammonia into excess water that had been added to the system in order to maintain sufficient moisture within the peat bed. Although the concentration of ammonia in the percolate actually decreased on day 29, when the high removal efficiency was achieved, (due to dilution of ammonia by the water), 22.95 mg ammonia emerged untreated in the percolate, which was 39 % of the total ammonia supplied to the filter. However there was also evidence of further nitrification because the pH decreased to pH 7.53, indicating that ratio of ammonia to nitrites/nitrates had changed. The concentration of ammonia in the percolate had also decreased. Even though there was evidence of nitrification the overall trend indicated that the nitrifier counts were decreasing. As the peat was inoculated with a mixed culture there is a possibility that there was an ammonia-oxidising species present that actively metabolised the ammonia but which was only a minor part of the overall nitrifier community. Also the media used in the MPN method for nitrifier enumeration was specifically for ammonia oxidisers of the genus *Nitrosomonas.* The method and media used may have underestimated the population as all *Nitrosomonas* species must have been capable of growth in that media and also there may have been other ammonia-oxidising genus present, which remained undetected (Underhill, 1990). The reduction in cell numbers could also have been due to sampling difficulties. As already stated nitrifiers are light sensitive and samples could only be obtained from the surfaces of the peat bed. Nitrification alternatively could have been due to heterotrophic organisms. In soil environments that are sub-optimal for autotrophic growth e.g. acid soils there are often heterotrophs present capable of nitrification. Chung *et al.* (1997) isolated a

heterotrophic *Arthrobacter oxydans* CH8 from livestock farming wastewater, which was capable of nitrifying ammonia in a biofilter. A marine bacterium, *Vibrio alginolyticus,* was used by Kim *et al.* (2000) to treat ammonia in a biofilter packed with an inorganic material (Fuyolite). Ammonia was supplied at concentrations varying from 152 mg m^{-3} to 911 mg m^{-3} at mass loads of 2.4 – 22.5 g N Kg^{-1} dry packing d^{-1} (0.12 – 1.14 g NH_3 Kg^{-1} packing h^{-1}). However nitrification by native peat heterotrophic organisms was unlikely as, as already stated, both Hartikainen *et al.* (1996) and Togashi *et al.* (1986) reported that native peat had no nitrification potential and they inoculated their biofilters. Nitrifying activated sludge was used as the inoculum by both researchers and therefore there was a mixed population of micro-organisms present. Neither of them followed the nitrifier population during biofilter operation. Yani *et al.* (1998) also inoculated the peat biofilter with activated sludge and on day 73 the nitrifier counts were high, at 6.8 x 10^7 cfu g^{-1} peat. The heterotrophic population was not enumerated. However, with such a high count of autotrophic organisms nitrification it was assumed that nitrification was due to autotrophs because they are much more efficient at ammonia conversion than heterotrophs (Kuenen *et al.*, 1988).

Even though ammonia was supplied to the biofilter on a regular basis for the remainder of stage 1 ammonia concentration in the percolate and the pH of the percolate did not increase, which was similar to the situation encountered by Togashi *et al.* (1986). They also reported NO_x-N accumulation on the peat in their biofilter, which was inoculated with nitrifying activated sludge, and supplied with 40 ppm (30.4 mg m^3) ammonia at a mass load of 0.16 g N Kg^{-1} dry peat d^{-1} (0.008 g NH_3 Kg^{-1} peat h^{-1}). Although nitrate accumulated and ammonia adsorbed by the peat did not increase the pH did not drop below pH 6.95 and they concluded that ammonia irreversibly trapped on the peat was oxidised to NO_x-N and then neutralised with NH_4-N to yield NH_4NO_x. A balance was achieved between incoming ammonia and nitrate production and therefore no adverse effects such as pH increase or decrease or high levels of ammonia accumulation occurred. Complete removal was accomplished for the 101 days of operation.

In this study the packing material possibly adsorbed excess ammonia that was not nitrified.

During stage 2 ammonia was supplied to the biofilter with higher inlet concentrations and higher mass loads than previously reported laboratory-scale biofilters used to treat ammonia. However the removal efficiency of ammonia remained high throughout the stage.

Removal was due to nitrification, which was confirmed by the presence of nitrites and/or nitrates in the percolate but there was also some accumulation of ammonia in the system. Until day 61 of stage 2 the concentration of nitrate in the percolate was unknown but the pH indicated that nitrates were continually produced. As ammonia was supplied to the system the concentration of ammonia in the percolate gradually increased however, even with the increase in ammonia concentration the pH of the percolate decreased to pH 5.95 (day 55). Acid production (in the form of nitrites and nitrates) occurred at a faster rate than base (in the form of ammonium) accumulated in the system and therefore there was a drop in pH. At the lower pH experienced on day 55 the nitrification was inhibited and thus the rate decreased and as a result the pH of the percolate increased again to pH 8.05 (day 57). Chung and Huang (1998) reported that the growth rate of *Nitrosomonas europaea* fell to 25 % of its maximum rate at pH 6.5 and stopped completely at pH 6.0. Pure cultures are more sensitive to environmental conditions than are free-living organisms according to Boch *et al.* (1991). Free-living *Nitrosomonas* can grow in the pH range of pH 5.8 – 9.5, which is the broadest range of all ammonia-oxidisers but it is expected that the rate of nitrification be much reduced at the lower pH values. Hartikainen *et al.* (1996) inoculated the peat bed with nitrifying activated sludge that contained 5×10^5 ammonia oxidisers L^{-1} and 4×10^6 nitrite oxidisers L^{-1}. Nitrate was produced when the pH of the peat was maintained at pH 6.0 but no nitrates were produced when the peat pH was maintained at pH 4.0. As expected from the studies of Chung and Huang (1998) and Hartikainen *et al.* (1996) at the lower pH values experienced on day 55 of this study the rate of nitrification was reduced. Therefore the rate of ammonia accumulation exceeded the rate of nitrate formation and the pH increased. Ammonia concentration, nitrate concentration and the pH of the percolate continued to increase gradually until day 116, which illustrated that ammonia was supplied to the system at a faster rate than it was metabolised indicating that the system was possibly overloaded with substrate. However, although there was some accumulation of ammonia in the system the removal efficiency remained high. The gradual increase in the ammonia concentration may not have been due to free ammonia

entering the system but due rather to incoming ammonia that served as a neutralising agent to the nitrates that were produced. Ammonia involved in the formation NH_4NO_3 dissociates in the presence of a basic solution e.g. ISA used with the ammonia ion selective electrode and was then detected with the free ammonia. Alternatively ammonia accumulation in the biofilter used in this research was possibly due to supply of ammonia outside the nitrification capacity of the microbes and would eventually lead to complete inhibition of nitrification. Togashi *et al.* (1986) recommended that the maximum safe inlet loading should be 70 % of the nitrifying capacity of the peat. And according to Kim *et al.*, (2000), the direct introduction of 200 $\mu L\ L^{-1}$ (152 mg m^{-3}) ammonia is inhibitory to nitrifiers and 1200 $\mu L\ L^{-1}$ (911 mg m^{-3}) is beyond their nitrification capacity. (The average inlet concentration supplied during stage 2 was 564 mg m^{-3}). According to Anthonisen *et al.* (1976) it is free ammonia in solution as opposed to total aammonia that is inhibitory to nitrification. Ammonia will be in solution as ammonium ion (NH_4^+) and as un-ionised ammonia (NH_3). Free ammonia in solution at concentrations of 0.1 – 1.0 mg L^{-1} is inhibitory to nitrite oxidising organisms and concentrations of 10 – 150 mg L^{-1} to ammonia oxidising bacteria. However Stuven *et al.* (1992) reported no inhibition of *Nitrobacter* by ammonium. Pure cultures of *Nitrobacter vulgaris* grown in media containing 200 mg L^{-1} nitrite supplemented with 180 mg L^{-1} ammonium were not inhibited but if mixed cultures of *Nitrobacter* and *Nitrosomonas* were used only 58% of the nitrite was oxidised to nitrate. Hydroxylamine produced during ammonia oxidation caused reduction of the nitrite to NO and N_2O and therefore only 58 % nitrite was available for oxidation.

The concentrations of ammonia in the percolate did not completely inhibit microbial activity as the pH decreased after day 116 indicating that acid was still produced. i.e. the nitrifiers were not completely inhibited indicating that. It was possibly that the ammonia detected in the percolate was not freely available to the micro-organism but instead was involved in the neutralisation of nitrates as previously mentioned. Although the removal efficiency was high, by day 116, both the fungal counts and nitrifier counts showed a decreasing trend. The heterotrophic bacterial counts were relatively stable at 10^4 cells g^{-1} peat at sample ports A and B and the counts increased exponentially between day 105 and day 116 at sample port C, closest to the ammonia inlet. The exponential increase in bacterial cells could have been due to an increase

in nutrient availability as the peat compacted under the harsh conditions it was exposed to in the biofilter. Although the fungal and nitrifier populations were relatively constant, there was evidence of some decrease, which may be attributed to environmental factors as opposed to ammonia toxicity. The pH of the percolate was greater than pH 7.00 from day 75 onwards, which would have a negative effect on fungal growth. As the biofilter conditions obviously favoured heterotrophic growth, they would have out-competed the slower growing autotrophic nitrifiers for available nutrients.

Between day 116 and day 137 the biofilter appeared to have reached steady state. Although ammonia was supplied with the same mass load and the same inlet concentrations as previous runs, the concentrations of ammonia and nitrate in the percolate remained constant, the pH was neutral and the removal efficiency was greater than 99 %. This indicated incoming ammonia was converted to nitrate, which was then neutralised with free ammonia to NH_4NO_x and subsequently utilised by heterotrophic organisms as a nitrogen source. However during that time the nitrifier counts decreased further, the fungal counts remained below 10×10^3 cfu g^{-1} peat. The only evidence of growth was a 3-fold increase in the heterotrophic bacterial counts at sample port A. Nitrification may have been due to heterotrophic organisms or undetected nitrifiers. Alternatively ammonia removal could have been due to adsorbance on to the packing material, which would not affect the ammonia concentration or pH of the percolate.

The peat bed was gently mixed on day 147 as already stated in the results section to minimise the development of holes that resulted from the regular removal of peat samples for microbial analysis. Both, the concentrations of ammonia and nitrate in the percolate increased significantly as a result of the disturbance to the peat bed (day 162) indicating that there were pockets in the peat bed where ammonia and its nitrified products were accumulated. There were possibly areas in the biofilter that were over-watered and other areas that dried out as a result of inefficient percolation of water from the sprinkler throughout the peat bed. Thus percolate analysis gives an overall indication of increasing and decreasing ammonia and nitrate values and of pH changes within the system, but as the bed is not uniform it has limited usefulness. Hartikainen *et al.* (1996) monitored the pH of their biofilter by extending electrodes up and down the column wall and by analysing the pH of the rinsing water. Both methods showed the same trend and therefore percolate analysis was assumed to be a

sufficient method for determining bed pH. Although it was considered that removal of ammonia by the biofilter had reached a steady state by day 116, there was considerable accumulation of ammonia in the system that was undetected, indicating that the biofilter was not operating as adequately as suspected from day 116.

Mixing the packing material disrupted the biofilm and heterotrophic cell numbers decreased at each sample port. Fungal counts were unaffected and they remained permanently low. Nitrifier counts actually increased after the peat bed was mixed, most predominantly at sample port C. Although mixing the peat bed disrupted the biofilm, the reduction in heterotrophic bacteria diminished the competition within the biofilter and thus allowed more rapid growth of the nitrifiers. Alternatively the increase in nitrifier numbers could have been due to a gap in ammonia supply at that time.

Overall the filter used in this experiment compared very favourably with other reported laboratory scale biofilters. The concentrations of ammonia supplied together with mass loads and empty bed contact times are summarised in Table 25

Table 25 Comparison of operating parameters of peat biofilters successfully used to treat ammonia

Author	Inlet conc. ($mg\ m^{-3}$)	EBCT (s)	Mass load ($g\ NH_3\ Kg^{-1}$ peat h^{-1})
Yani *et al.* (1998)	19.4 – 153	11 – 165	0.0067 – 0.18
Hartikainen *et al.* (1996)	0.5 – 14	28	0.00075 – 0.021
Martin *et al.* (1996)	20 – 30	7 – 25	unknown
Togashi *et al.* (1986)	30	unknown	0.0081
Biofilter used in this research	3 – 47 69 – 800 1740 – 2900	37 42 – 47 47	0.0005 – 0.0077 0.0093 – 0.12 0.235 – 0.392

The inlet concentrations supplied to the biofilter used in this investigation during stage 1 was comparable to those supplied by Hartikainen *et al.* (1996), Martin *et al.* (1996) and Togashi *et al.* (1986). Both Hartikainen *et al.* (1996) and Togashi *et al.* (1986) achieved complete removal of ammonia from the waste gas stream under the conditions described in Table 25. Hartikainen *et al.* (1996) removed the ammonia

with an EBCT that was almost 10 s shorter than was required in this research and the mass loads they applied to their peat bed was higher than what was applied to the peat bed investigated during this research. However in this research similar high removal efficiencies were achieved when the inlet concentrations and mass loads were substantially stepped up without requiring an increase in EBCT. Hartikainen *et al.* (1996) could not maintain a high removal efficiency when ammonia was supplied at an initial inlet concentration of 45 mg m^{-3}, at a corresponding mass load of 0.09 g NH_3 Kg^{-1} peat h^{-1}, with an EBCT of 21 s. The removal efficiency began to decline within 4 days. The experiment was run for 35 days and it was found that the nitrate concentration in the percolate increased for 2 weeks and then stabilised. At the same time the pH increased to pH 9.00, indicating that ammonia accumulated in the system. For the remainder of the experiment there was a significant increase in nitrite concentrations in the percolate indicating that the nitrite oxidisers were inhibited by the high concentrations of ammonia that had accumulated in the system. Nitrite oxidising bacteria are more sensitive to high concentrations of ammonia than the ammonia oxidising organisms (Antonisen, 1976). Likewise Togashi *et al.* (1986) could not achieve complete removal of ammonia when the inlet concentration was maintained at 40 ppm (30.4 mg m^{-3}) but the mass load was increased from 0.16 g N Kg^{-1} dry peat d^{-1} (0.0081 g NH_3 Kg^{-1} peat h^{-1}) to 0.32 g N Kg^{-1}dry peat d^{-1} (0.016 g NH_3 Kg^{-1} peat h^{-1}). Initially nitrates accumulated rapidly on the peat and ammonia adsorbed by the peat fluctuated, with an increasing trend. Ammonia accumulation inhibits nitrification and therefore removal became unstable. The load of ammonia was outside the nitrification capacity of the peat, which lead to ammonia accumulation and eventually complete inhibition of nitrification. Although Martin *et al.*, (1996) claimed that ammonia was eliminated from a waste gas stream with an inlet concentration of 25 mg m^{-3}, the removal efficiency was less than 60 % within 20 days of operation. Nitrates were not detected throughout the experiment although the autotrophic nitrifiers were estimated at $10^5 - 10^6$ cfu g^{-1} peat. The heterotrophic biomass however increased significantly during the period of high removal, which was due to physico-chemical reactions and then stabilised. Additional nutrients e.g. phosphates were added during biofiltration and it was found that ammonia removal was higher when the peat bed was treated with additional nutrients. The addition of nutrients would have encouraged heterotrophic growth and therefore introduced additional competition for the autotrophic organisms.

Yani *et al.* (1998) operated at higher inlet concentrations than those reported by the other authors. The inlet concentrations used were similar to stage 1 and the earlier part of stage 2 in this research. However the mass loads were equivalent to and higher than the mass loads supplied to the biofilter during stage 2 of this research, which is higher than any reported mass loads applied to a peat based laboratory scale biofilter for the treatment of ammonia. At the higher mass loads of 0.18 g NH_3 Kg^{-1} peat h^{-1} their removal efficiency decreased to 80 % and the load was immediately reduced to achieve steady removal again.

No nutrients were supplied to biofilter in this research, however the heterotrophic population survived well without requirements for additional substrates. Although by the end of the experiment both fungal and nitrifier populations had decreased, it was more likely due to the surrounding environment than lack of nutrients. Martin *et al.* (1996) supplied nutrients and found a higher degree of ammonia removal and a larger biomass when additional phosphates were supplied to the biofilter.

Very high concentrations of ammonia were supplied to the biofilter during stage 3 to examine the effects of such concentrations on both the peat bed and on the microbial population. Overall and the peat compacted and neither the fungal nor autotrophic nitrifiers survived under the harsh environmental conditions encountered during stage 3. The heterotrophic bacterial populations were not affected by the high loads of ammonia supplied during stage 3.

Although in general terms the removal efficiency was high during stage 3, there was a decreasing trend. This was due to the fact that the peat bed was compacting under the higher loads of ammonia supplied in stage 3. Ammonia is a very corrosive gas and it was supplied to the peat at very high concentration, which enhanced compaction. Indigenous micro-organisms also mineralised the peat as it is an organic material that can be used as nutrient source, which added to the compaction of the packing material (Leson and Winer, 1991). The bacterial cells, although they were relatively constant throughout stage 3, showed some indication of decline towards the end of biofilter operation at sample ports A and B, which may have been due to exhaustion of the peat material. Holes had also developed in the bed where samples had been removed and not replaced. Air follows the path of least resistance and therefore residence time was reduced and the air emerged partially untreated. The volume of percolate decreased during stage 3 although the moisture was maintained

as it had been for the initial stages. As the peat bed became more compact the water sprinkled onto the biofilter did not percolate uniformly through the bed. The water was probably retained by the smaller particle sizes (as a result of compaction) and thus clogged pores and reduced areas available to degrade the incoming pollutant and hence aided reducing the residence time of the pollutant in the packed bed. The concentration of ammonia and nitrate in the percolate decreased over time and it emerged immediately after the filter was sprinkled with water indicating that it was again running along the path of least resistance and therefore the entire bed was not uniformly moistened. The water washed irreversibly trapped ammonia and nitrates from the same areas of the packed bed. As the concentrations of both ammonia and nitrate decreased over time the incoming ammonia and it's nitrified products (if any) were retained in some part of the filter that the water did not percolate through. Once the bed material becomes compact the percolate can not be used as a fair indicator of what happens in the filter. Also an alternative method for moistening biofilters should be investigated as the non-uniformity of the packing prevents even distribution of water throughout the bed.

As fresh packing material was added to the top of the biofilter and the nitrifier and fungal counts had decreased during stage 3 it was deemed necessary to inoculate the peat with activated sludge again. Re-inoculating the filter during stage 3 had no effect on the performance of the biofilter. The removal efficiency, which was already high but decreasing continued to decrease. There was a temporary increase in the nitrifier counts but ultimately they could not survive the harsh biofilter conditions, which may have been due to accumulation of high concentrations of ammonia that inhibited the organisms or due to competition with the heterotrophic organisms. As the heterotrophic bacteria were already actively growing the effect of inoculation of that population was difficult to ascertain. Due to the high pH of the system the fungal cells died, growth was not initiated even after inoculation. There was a temporary increase at sample port A 24 hours after inoculation but the fresh inoculum did not survive in the environment provided by the biofilter.

At the end of stage 3 the material had compacted, the removal efficiency was decreasing and fungal and autotrophic organisms could not survive in the filter. This may have been due to exhaustion of filter material or oxygen transfer problems due to overwet sections in the peat bed or due to toxicity effects of ammonia that was accumulating in some parts of the filter that remained undetected.

5.0 MAIN FINDINGS

- a laboratory-scale biofilter was successfully designed and operated
- gaseous ammonia was removed by the biofilter at inlet concentrations of 13.9 mg m^{-3}, 564.8 mg m^{-3} and 2226 mg m^{-3} with an overall average removal efficiency greater than 90 %
- shock-loadings and shut-down periods had no adverse effects on removal efficiency
- the elimination capacity of the biofilter increased linearly with increasing mass load
- while the heterotrophic bacteria, the nitrifying bacteria and the fungi all grew initially in the biofilter, the nitrifiers and the fungi did not survive the high loads of ammonia
- up to 40% of the ammonia supplied was adsorbed by the biofilter
- towards the end of biofiltration the peat bed compacted indicating that peat is not suitable for the biofiltration of high concentrations of ammonia

6.0 BIBLIOGRAPHY

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