BIOLOGICAL DENITRIFICATION - CONTROL OF ADVERSE SIDE EFFECTS

FINAL CONTRACT REPORT TO DEPARTMENT OF THE ENVIRONMENT

DoE 2699-SW

November 1991
BIOLOGICAL DENITRIFICATION - CONTROL OF ADVERSE SIDE EFFECTS

FINAL CONTRACT REPORT TO DEPARTMENT OF THE ENVIRONMENT - MARCH 1991

Report No: DoE 2699-SW

November 1991

Authors: T Hall

Contract Manager: R A Hyde

Contract No: 9807

Contract Duration: August 1988 to March 1991

DoE Reference No: PECD 7/7/303

Any enquiries relating to this report should be referred to the Contract Manager at the following address:

WRc Swindon, P O Box 85, Frankland Road, Blagrove, Swindon, Wiltshire SN5 8YR.
Telephone: (0793) 511711.
PREFACE

In order to meet drinking water quality standards, treatment plants to remove nitrate will need to be installed at many sites over the next few years. Biological denitrification processes have been demonstrated to be feasible at full-scale. However, means of controlling nitrite and residual carbon source concentrations need to be established to maintain satisfactory treated water quality.

In August 1988, the Department of the Environment placed a contract (PECD 7/7/303) with WRc for a study to help establish reliable monitoring techniques for carbon sources and recommendations for plant operation to minimise nitrite concentrations in the treated water. The study ended 31 March 1991.

This final report summarises the work undertaken.
Nitrate removal treatment processes will be needed at many sites within the next few years to achieve drinking water quality standards. Biological denitrification processes have been demonstrated to be feasible at full-scale for this application. However, means of controlling nitrite production and carbon source residual concentration are needed to maintain satisfactory treated water quality.

The objectives of this work were to establish reliable monitoring techniques for the carbon sources used in biological denitrification, and recommendations for operating conditions to minimise nitrite concentrations in the treated water.

A volatile organics monitor developed by WRc, and a gas chromatography technique using head-space vapour injection, have been shown to be capable of achieving the desired detection limits for methanol and ethanol, but not for acetic acid. Biosensor techniques also show promise for monitoring and control of carbon source residual concentrations, but costly development work would be needed to produce a prototype system for further investigation.

Control of nitrite residual can be achieved through overdosing of carbon source to establish nitrate limited operation. Excess carbon source can be removed by the development of biological activity in post-denitrification conventional water treatment processes.

Report No: DoE 2699

iii
5.2 GUIDELINES FOR PROCESS SELECTION, DESIGN AND OPERATION

5.2.1 Process selection

5.2.2 Design and Operation of BFB plants

SECTION 6 - CONCLUSIONS AND RECOMMENDATIONS

REFERENCES

APPENDICES

A PRINCIPLES OF ENZYME TEST KITS FOR ETHANOL AND ACETIC ACID
B INFORMATION ON ENZYME BASED MONITORING PROCEDURE FROM BIRD ENGINEERING
C SUMMARY OF REPORT ON BIOSENSORS FROM CRANFIELD BIOTECHNOLOGY CENTRE
D RESULTS FROM MATHEMATICAL MODELS.
SECTION 1 - BACKGROUND

To meet the standard set in The Water Supply (Water Quality) Regulations 1989, treatment processes for nitrate removal will need to be installed at many sites over the next few years. The two types of process currently favoured for this application are ion exchange and biological denitrification. The latter is likely to offer cost advantages over ion exchange for treatment of surface waters or for larger groundwater sources (above 20ML/d).

Biological denitrification relies on the ability of naturally-occurring bacteria to use nitrate for respiration in the absence of dissolved oxygen, reducing the nitrate to nitrogen gas:

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]

Many types of biological denitrification processes have been developed throughout Europe, differing in the type of physical support material used for bacterial attachment, and the energy source used to encourage bacterial growth. The processes are either autotrophic, in which the bacteria obtain their energy from oxidation of inorganic chemicals (hydrogen or sulphur), or heterotrophic, when an organic compound (e.g. methanol) is used as a bacterial food source.

In the UK a heterotrophic biological fluidised bed (BFB) process has been developed and evaluated at full-scale\(^{(1)}\). This process used sand, fluidised by upflow of the water being treated, as the physical support for bacterial growth. Methanol, ethanol or acetic acid were investigated as the carbon food source. Whilst the process was generally successful at achieving the design denitrification, two operational aspects of the work were identified as requiring attention in order to improve the process. These were:

- production of nitrite,
- monitoring of carbon source concentration in the treated water.
Nitrite (NO₂⁻) is an intermediate in the denitrification pathway. At times the nitrite concentration in the denitrified water was as high as 2mg NO₂N/l. The standard prescribed in the regulations for nitrite is 0.03mg NO₂N/l (0.1mg NO₂⁻/l). Nitrite can be readily oxidised by chlorine back to nitrate, but this impairs the efficiency of the denitrification process.

The concentration of carbon source remaining in the denitrified water needs to be rigorously controlled because of the implications on human health and the risk of bacterial aftergrowth in the distribution system.

This report describes work carried out under contract to the Department of the Environment aimed at investigating ways of controlling nitrite production during denitrification, and assessing techniques for monitoring carbon source residual concentrations in the treated water. The emphasis of the work is on BFB denitrification, although some of the information given, particularly that on carbon source monitoring, is relevant to other types of heterotrophic process. The report also reviews biological denitrification processes currently under investigation or in operation and provides guidelines for process selection, design and operation.
SECTION 2 - OBJECTIVES

1. To establish reliable monitoring techniques for the carbon sources used in biological denitrification.

2. To establish recommendations for plant operating conditions to minimise nitrite concentrations in denitrified water.
SECTION 3 - CARBON SOURCE MONITORING

3.1 GENERAL CONSIDERATIONS

The requirements of a continuous monitoring system for biological denitrification carbon sources are:

- it must be capable of monitoring methanol, ethanol or acetic acid concentrations of 1mg/l or less,

- it should be reliable enough to be left unattended for periods of two or three days,

- it should be robust and simple enough to allow maintenance by staff without high levels of technical training.

The types of monitoring systems that have been considered or previously used for this purpose are:

(i) An autoanalyser system for methanol determination in discrete samples or for continuous monitoring.

(ii) Gas chromatography (GC).

(iii) Enzyme-based techniques.

(iv) Electrochemical (fuel-cell) methods.

The autoanalyser system for methanol determination\(^2\)\(^,\)\(^3\) was used for monitoring of the full-scale BFB denitrification plant\(^1\). It can provide a suitably low limit of detection (0.2mg/l) but requires a high level of skilled attention, and one of the objectives of this work was to seek alternative monitoring techniques for methanol.
3.2 GAS CHROMATOGRAPHY

Gas chromatography (GC) has been used to measure ethanol and acetic acid concentrations in samples taken for the purposes of monitoring and control of the full-scale BFB denitrification plant, with detection limits of 0.8mg/l\(^1\). However, a much lower detection limit of 0.11mg/l has been reported\(^2\) using more stringent GC conditions, with direct aqueous injection of samples.

No attempts have been made to use GC for continuous monitoring of the denitrification plant. GC equipment is available which will allow automatic injection of samples at regular intervals, and such equipment has been used in Germany for monitoring and control of a process for removing chlorinated hydrocarbons from groundwater\(^4\).

Discussions were held with Siemens plc regarding the use of continuous gas chromatography for monitoring of methanol, ethanol or acetic acid. Proprietary equipment is available which could be used for this purpose. According to the manufacturers, a technique involving analysis of head space vapour should be capable of achieving detection limits below 1mg/l for the carbon sources of interest. GC using head space vapour analysis would be more suitable than direct aqueous injection of samples because it would overcome the need for a rigorous sample clean-up procedure required to prevent rapid fouling of the GC column.

The cost of the equipment in 1990 was approximately £20,000. It is fully automatic and is claimed to have a low maintenance requirement. It is, however, likely to need at least a once-weekly check by a chemist or suitably trained operator to ensure that it is functioning correctly.

Enquiries were made concerning the possibility of renting an instrument for testing. Siemens were only prepared to do this if there was a high probability of a sale of the instrument at the end of the rental period, partly because each instrument is, to some extent, tailored to a particular application. No further work could therefore be carried out
to test the instrument for monitoring of biological denitrification. Siemens would, they claimed, be prepared to guarantee the performance for this application to any potential purchaser, based on the information provided to them during our discussions and their own experience. However, some experimental work was carried out to investigate the sensitivity of GC for detection of methanol, ethanol and acetic acid in head space vapour.

The experimental work was carried out using a head-space sampling device fitted to a standard Perkin Elmer gas chromatograph. The sample is heated to 60°C and the head space vapour is injected into the GC. The results for solutions of methanol and ethanol at concentrations in the range 1 to 10mg/l (in tap water) are shown on Figure 1 in the form of a calibration graph of GC peak height against concentration. These results suggest that the technique is suitable for monitoring methanol and ethanol concentrations down to 1mg/l or less. The Siemens instrument involves air-purging of the sample as well as heating, which would further increase the head-space concentrations and detection limits compared with the method used in this investigation. The method was unsuccessful for detection of acetic acid at concentrations up to 200mg/l. Some improvement in sensitivity may have been obtained by reducing the pH of the solution. However, it is unlikely that the degree of improvement needed to achieve a detection limit of 1mg/l would have been possible.

3.3 ENZYME-BASED TECHNIQUES

Two types of enzyme-based techniques have been investigated:

- test kits designed for measuring ethanol and acetic acid concentrations in food and drinks,

- biosensors for methanol, ethanol and acetic acid.
FIGURE 1:

GC HEADSPACE METHOD RESULTS
FOR METHANOL AND ETHANOL

Peak Height (mm)

Concentration (mg/l)
3.3.1 Enzyme Test Kits

The test kits are marketed by the Boehringer Corporation. They were investigated as a means of measuring ethanol and acetic acid concentrations in discrete samples, and also to assess their potential for development into an automated method. The techniques are described in Appendix B. They are based on the spectrophotometric determination of biochemical reaction products resulting from enzymatic breakdown. The limit of detection is governed by the minimum absorbances that can be accurately measured. The methods recommend that the absorbance differences between the samples and blanks should not be less than 0.1 absorbance units. Using spiked river water samples, this limit was reached at concentrations of 5mg/l and 7mg/l respectively for ethanol and acetic acid, using a 4cm light path. These detection limits would be unsatisfactory for the purpose of plant monitoring and control.

However, a Dutch company, Bird Engineering, claims to have improved the sensitivity of a similar enzyme based technique and have started developing a monitor capable of detecting ethanol at concentrations down to 0.5mg/l. Information provided by this company is given in Appendix D.

3.3.2 Biosensors

A biosensor is an analytical system in which an electrical signal is produced in response to a biochemical reaction, the extent of the reaction governing the magnitude of the signal. They consist of immobilised biological material or biocatalyst (e.g. enzymes) in contact with a suitable transducer device, which converts a biochemical signal into a quantifiable electrical signal. Selection of suitable biological material can make biosensor systems specific for individual compounds. Such a system could offer advantages over chemical analysis systems with respect to ease of operation and maintenance, particularly because dosing of reagents to the sample stream would be unnecessary.

Cranfield Biotechnology Centre was sub-contracted to carry out a review of biosensor technology with respect to its application to monitoring
methanol, ethanol or acetic acid in biological denitrification. The report produced by Cranfield indicates that methanol, ethanol or acetic acid could be monitored continuously, with suitable detection limits (1mg/l or less) using a biosensor system based on immobilised enzymes in a column through which a small sample stream is allowed to flow. The cost of such a system, once developed, is likely to be relatively small at around £10,000. However, the development cost for a prototype system, including 1 man-year of skilled labour, is likely to be around £50,000 and is therefore beyond the scope of this contract. The report, however, gives recommendations and contacts for carrying out this development work, should this be required.

3.4 ELECTROCHEMICAL METHODS

Analytical instruments for alcohol determination based on fuel cells are commercially available\(^5,6\). In a fuel cell, a fuel (alcohol) is supplied to one electrode and an oxidant (e.g. oxygen or air) to another. Oxidation of the fuel generates a voltage in proportion to the amount of alcohol present. Measurements are made on head-space vapour produced by heating of the sample under test. The limit of detection is governed by the sensitivity of the fuel cell and the volatility of the compound being analysed. The method is normally used for determination of ethanol in blood samples or drinks, at concentrations much higher than those of interest for this work. However, a proprietary fuel cell instrument has been used by WRc in the past to measure methanol concentrations down to 2mg/l in discrete samples from biological denitrification plants, although the accuracy of measurement at this level was poor (+100%). Attempts were also made to develop this procedure into a continuous monitoring device for methanol, without success.

WRc Medmenham has developed a monitor for volatile organic compounds (VOCs) in water based on a fuel cell. Experiments have been carried out to investigate the use of this equipment for monitoring of biological denitrification carbon source residuals. The equipment, illustrated on Figure 2, consists of a heated cell containing an electrochemical sensor specific to the type of organic compound under investigation. The water
Figure 2: VOC Monitor
under test flows through the cell and is purged with recycled air to remove any volatile components. The sensor detects the organic compound in the head-space vapour above the water, and produces an output voltage proportional to concentration. The voltage is amplified and recorded.

Figure 3 shows results for the use of the VOC monitor on water samples spiked with ethanol or methanol at concentrations in the range 0.4mg/l to 80mg/l. The instrument responded similarly for both compounds. The response obtained suggests that a detection limit of 1mg/l should be easily achieved for both compounds. Similar tests were carried out using acetic acid, but the response of the instrument with all the available types of sensor was poor. Reducing the pH to convert acetate to the more volatile acetic acid did not significantly improve the sensitivity.

Further work needs to be carried out to investigate the performance of the instrument under continuous operation, particularly with regard to stability of the output signal. However, at this stage the instrument shows promise not only with respect to detection limits for ethanol and methanol, but also for its likely ease of operation and cost (probably less than £5,000).
Figure 3: V.O.C. monitor results for ethanol and methanol.
SECTION 4 - NITRITE PRODUCTION

Investigations of nitrite production in denitrifying systems have involved:

- laboratory batch tests,
- analysis of plant operating data,
- pilot plant trials,
- mathematical modelling.

4.1 LABORATORY BATCH TESTS

Attempts were made to develop a laboratory batch test to investigate factors influencing nitrite production, such as temperature, carbon source concentrations, nitrate concentrations and biomass concentrations.

The apparatus involved is illustrated in Figure 4. Biomass sludge samples from the pilot plant were stood in a 5 l container in a water bath until the desired temperature was reached. Denitrification was initiated by adding nitrate and carbon source to the stirred sample. The progress of the denitrification reaction was monitored using a nitrate ion-selective electrode. Samples of sludge were taken at intervals for analysis of nitrate, nitrite and carbon source.

An example of the results obtained (Table 1) indicated that nitrite accumulated in a biomass sample dosed with insufficient methanol for complete denitrification (i.e. underdosed). Once the methanol is used up, the nitrite cannot be removed by the bacteria. However when further methanol is added at this stage to overdose the system with excess methanol for complete denitrification, the nitrite is reduced to acceptable levels. This is consistent with result from plant operation and is in agreement with enzyme kinetic model predictions of denitrification\(^{(7)}\).
Figure 4: Laboratory Denitrification Batch Test Apparatus
Table 1 - Example of results from laboratory batch test

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>17</th>
<th>25</th>
<th>35</th>
<th>38</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3$N (mg/l)</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>NO$_2$N (mg/l)</td>
<td>0.1</td>
<td>0.15</td>
<td>0.18</td>
<td>0.22</td>
<td>0.55</td>
<td>0.55</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Methanol (mg/l)</td>
<td>69</td>
<td>&lt;0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5*</td>
<td></td>
</tr>
<tr>
<td>* 5mg/l added</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Many practical problems were encountered in the development of this batch test. It was found that duplicate tests carried out consecutively within a one hour period under nominally identical conditions gave widely differing problems of nitrite production. These differences appeared to be too large to have resulted as an artefact from the method, and suggested that the denitrification reaction is highly unstable with respect to nitrite production. This is consistent with results from plant operation; nitrite production by BFB denitrification can increase substantially over relatively short periods (e.g. 24 hours) without any other apparent change in operating conditions when the plant is operated with underdosing of carbon source.

This instability and non-reproducibility of the batch test meant that attempts to develop and use the test procedure had to be abandoned in favour of other approaches to the investigation of nitrite production.

4.2 ANALYSIS OF PLANT OPERATING DATA

During operation of the full-scale BFB denitrification plant and the pilot plants, samples were taken at depth intervals down the fluidised bed, and analysed for methanol, nitrate, nitrite and volatile solids (VS, a measure of the biomass concentration on the fluidised sand). Results of these profile samples were used to calculate methanol removal rates, but no detailed analysis was carried out of the rates of nitrite production within the bed. These profile data have now been re-assessed with regard to nitrite kinetics using the procedure described below.
Samples of water and sand were taken at 0.5 to 1m intervals from the bottom of the fluidised bed. Typical analysis results for two points in the bed might be:

<table>
<thead>
<tr>
<th>DISTANCE FROM BASE (m)</th>
<th>NO$_3$N (mg/l)</th>
<th>NO$_2$N (mg/l)</th>
<th>VS (g/l)</th>
<th>MEAN VS (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.0</td>
<td>0.5</td>
<td>12.0</td>
<td>13.5</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
<td>1.0</td>
<td>15.0</td>
<td></td>
</tr>
</tbody>
</table>

Biological denitrification can be considered basically as a two-stage process for the purposes of modelling:

$$\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2$$

Denitrification models based on enzyme kinetics have shown that accumulation of nitrite occurs when the rate of nitrate reduction (or nitrite production) exceeds the rate of nitrite reduction (or nitrite removal). These rates (expressed per unit VS) can be calculated from profile results. Assuming that during denitrification all nitrate removed goes via nitrite, the nitrite production and removal rates between two points in the bed are calculated from the changes in nitrate and nitrite, the bed volume, the mean VS concentration and the flow rate. For example, using the values shown above with a 1m$^2$ area of bed (i.e. 1m$^3$ bed volume) and an upflow rate of 20m/h, the rate of nitrite production would be calculated as:

\[
\text{nitr} \text{e production rate} = \frac{\Delta \text{NO}_3N \times \text{flow rate (m}^3/\text{h)}}{\text{mean VS \times bed volume}}
\]

\[
= \frac{(10 - 5) \times 20}{13.5 \times 1}
\]

\[
= 7.5\text{g NO}_2N/\text{KgVS.h}
\]
where $\Delta \text{NO}_3\text{N} = \text{nitrate at lower point} - \text{nitrate at upper point}$. 

The rate of nitrite removal is:

\[
\text{nitrite removal rate} = \frac{(\Delta \text{NO}_3\text{N} + \Delta \text{NO}_2\text{N}) \times \text{flow rate}}{\text{mean VS} \times \text{bed volume}}
\]

\[
= \frac{[(10 - 5) + (0.5 - 1.0)] \times 20}{13.5 \times 1}
\]

\[
= 6.7 \text{g NO}_2\text{N/KgVS.h}
\]

The accumulation rate is the production rate minus the removal rate (i.e. 0.8g NO$_2$N/KgVS/h for the above example). The results are expressed per unit VS to allow for the considerable differences in VS concentrations encountered during plant operation.

This approach has been used for over 50 sets of profile results from the pilot scale and full-scale plant. Possible correlations have been investigated between operational conditions and rates of nitrite production, removal and accumulation within the bed. Calculations were carried out for the bottom 1m of bed and the 1 to 2m region separately. The operational conditions considered were:

- water temperature,
- mean VS concentration in the bottom 1m and between 1 and 2m,
- mean NO$_3$N concentration in the bottom 1m and between 1 and 2m,
- mean methanol concentration in the bottom 1m and between 1 and 2m,
- mean NO$_2$N concentration in the bottom 1m and between 1 and 2m,
- NO$_2$N concentration in the denitrified water leaving the bed.

No significant correlations were found between operating conditions and rates of nitrite production, removal or accumulation within the bed. The highest correlation coefficient ($r = 0.68$) was between the accumulation of nitrite in the bottom 1m of the bed and final nitrite concentration in the treated water. This suggests that the reaction rates in this region
of the bed are important in relation to nitrite concentrations in the final water but does not give any information useful for plant operation or control.

4.3 PILOT PLANT TRIALS

Operational of the full-scale BFB denitrification plant indicated that overdosing of carbon source, to achieve complete denitrification, could prevent the occurrence of high nitrite concentrations in the denitrified water. A detailed investigation of this approach was not possible on the full-scale plant because of operational constraints. Pilot plant trials were subsequently undertaken to investigate:

- the feasibility of long-term operation with overdosing of carbon source, and the effect on nitrite residual,

- removal of excess carbon source by conventional water treatment processes.

The work was carried out as part of the WRc subscription research programme during the period 1987 to 1989, but additional monitoring of the plant was carried out as part of this DoE contract. The results of the work are summarised here.

The pilot plant consisted of two 0.3m diameter BFBs operated in parallel at an upflow rate of 20m/hour. Methanol was used as the carbon source. The required dose is governed by the nitrate and dissolved oxygen concentrations in the feed water. The dose to one bed was controlled to remove 80 to 90% of the influent nitrate, leaving about 2mgNO₃N/l in the denitrified water (i.e. operation under carbon-limited or underdosed conditions). The other bed was overdosed with methanol to achieve full denitrification (nitrate limited operation). The water from the overdosed bed was then aerated and subjected to further treatment by pilot scale floc blanket clarification and/or rapid gravity filtration.
No consistent differences were found between the overdosed and underdosed beds with respect to nitrite concentrations in the lower regions (up to 2m from the base) of the beds. Analysis of the data for rates of nitrite production and removal, using the method described in Section 4.3, did not reveal any significant differences between the beds, or any significant correlations with other operating conditions, consistent with the findings for the full-scale plant.

However, there were significant differences between the beds with respect to nitrite concentrations in the upper regions of the bed. The underdosed bed often produced denitrified water high in nitrite (up to 1.5mgNO\textsubscript{2}N/l), whereas the nitrite concentration from the overdosed bed never exceeded 0.1mgNO\textsubscript{2}N/l during normal operation. The reason for this is illustrated by the profile data shown in Table 2. In the underdosed bed the methanol is used up, with nitrite (and nitrate) remaining in the denitrified water. In the overdosed bed, once the nitrate has disappeared, there is still sufficient methanol left for the bacteria to remove the remaining nitrite.

<table>
<thead>
<tr>
<th>HEIGHT ABOVE BASE OF BFB (m)</th>
<th>UNDERDOSED BED (mg/l)</th>
<th>OVERDOSED BED (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO\textsubscript{3}N</td>
<td>NO\textsubscript{2}N</td>
</tr>
<tr>
<td>0</td>
<td>13</td>
<td>0.12</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.48</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.60</td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>0.42</td>
</tr>
</tbody>
</table>

The objective for the overdosed bed was to leave up to 5mg/l methanol in the denitrified water, which would then need to be removed by subsequent biological treatment. The intention was to develop aerobic bacterial activity in a floc blanket clarifier and/or rapid gravity filters, which would remove excess methanol from the reaerated water.
Examples of the results for methanol removal are shown in Table 3, based on average values for samples taken at intervals over a 2 hour period immediately after filter backwashing. These results indicate that a methanol excess of up to 5mg/l can be removed by conventional treatment processes subsequent to BFB denitrification. This is likely to result from accumulation of bacteria (carried over from the BFB) within the floc blanket or filter. Regular sampling of the plant over the whole period of operation indicated that similar removal could be obtained at water temperatures of below 10°C.

Table 3 - Methanol removal in conventional treatment processes

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>MEAN METHANOL (mg/l) IN:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEED</td>
</tr>
<tr>
<td>Rapid gravity filtration (8/16 grade sand)</td>
<td>1.7</td>
</tr>
<tr>
<td>Rapid gravity filtration (16/30 grade sand)</td>
<td>1.8</td>
</tr>
<tr>
<td>*Chemical coagulation and rapid gravity filtration (8/16 grade sand)</td>
<td>4.8</td>
</tr>
<tr>
<td>*Chemical coagulation and floc blanket clarification</td>
<td>4.8</td>
</tr>
<tr>
<td>*Chemical coagulation, floc blanket clarification and rapid gravity filtration (8/16 grade sand)</td>
<td>4.8</td>
</tr>
<tr>
<td>Water temperature 16°C</td>
<td></td>
</tr>
<tr>
<td>* Chemical coagulation using Ferric Sulphate</td>
<td></td>
</tr>
</tbody>
</table>

4.4 Mathematical Model

4.4.1 Description

A computer mathematical model of biological denitrification was developed as a means of investigating the biochemical factors that can influence nitrite production. The model uses standard Monod biochemical kinetics,
adapted for application to a biological fluidised bed system (or any other system which is essentially plug flow). Calculations are carried out stepwise for predetermined time intervals progressing up the bed. The inlet nitrate, nitrite and methanol concentrations are used, together with biomass concentrations and kinetic parameters, to calculate the concentration changes over a small step up the bed. The new concentrations are then used as the inputs for the next step, and so on, for a total of 100 steps up the bed. The output from the model is a series of concentrations of nitrate, nitrite and methanol, representing a profile over the whole depth of the bed.

The logic for the model is summarised in Figure 5. Details of the calculations for each step are as follows:

(i) The specific growth rates of bacteria (g.bacteria/g.bacteria. hour) on nitrate and nitrite are calculated from:

\[
\mu_1 = \mu_{1 \ max} \frac{[NO_3]}{[NO_3] + k_{NO3}} \cdot \frac{[MeOH]}{[MeOH] + k_m}
\]

\[
\mu_2 = \mu_{2 \ max} \frac{[NO_2]}{[NO_2] + k_{NO2}} \cdot \frac{[MeOH]}{[MeOH] + k_m}
\]

where \( \mu_1 \) = specific growth rate on nitrate
\( \mu_2 \) = specific growth rate on nitrite
\( \mu_{1 \ max} \) = maximum specific growth rate on nitrate
\( \mu_{2 \ max} \) = maximum specific growth rate on nitrite
\( [NO_3] \) = nitrate concentration (mg N/l)
\( [NO_2] \) = nitrite concentration (mg N/l)
\( [MeOH] \) = methanol concentration (mg/l)
\( k_{NO3} \) = half-rate constant for nitrate (mg N/l)
\( k_{NO2} \) = half-rate constant for nitrite (mg N/l)
\( k_m \) = half-rate constant for methanol (mg/l)
(ii) From the individual specific growth rates on nitrate and nitrite, the methanol consumption is calculated based on the yield (g.bacteria produced/g.methanol).

Methanol consumption = \[ \frac{\text{specific growth rate} \times \text{biomass concentration}}{\text{Yield}} \]

(iii) The changes in nitrate and nitrite concentrations are calculated from the individual methanol consumptions for growth on nitrate and nitrite, based on the stoichiometry between methanol, nitrate and nitrite. The concentrations at the end of each step are:

\[
\begin{align*}
nitrates &= \text{initial nitrate} - \text{nitrate removed} \\
nitrite &= (\text{initial nitrite} + \text{nitrite produced}) - \text{nitrite removed}
\end{align*}
\]

where nitrite (N) produced = nitrate (N) removed.

(iv) The calculations outlined above allow for only two types of bacteria - those which convert nitrate to nitrite, and those which convert nitrite to nitrogen. It is assumed that all nitrate removed is reduced to nitrite, which is then released to the surrounding water for use by the nitrite reducing bacteria. The model also allows for a third bacterial type (or reaction mechanism) which can carry out both steps, nitrate to nitrite to nitrogen, without releasing nitrite. The calculation involves introducing a factor which represents the proportion of this third type of bacteria; the nitrite production in each step is calculated from the nitrate removed multiplied by this factor. It has been assumed initially that the other two types of bacteria are present in equal amounts.

Values for methanol and nitrate half-rate constants (\(k_m\) and \(k_{NO3}\)) were obtained from the literature\(^8\) and, because these do not have a major influence on the calculations, were kept constant. A value for yield obtained from plant operation was used. The
model was used to investigate the effects of changes in the variables, particularly those concerned with the kinetics ($\mu_{1\text{max}}$, $\mu_{2\text{max}}$ and $k_{\text{NO}_2}$), on nitrate profiles through the bed.

The model does not take into account dissolved oxygen (DO) removal, and therefore represent only the anoxic region of the bed above the point where all the DO has been removed.

Enter:
- feed water concentrations of nitrate, nitrite and methanol;
- biomass concentrations;
- kinetic constants;
- time increment per step.

Calculate specific growth rates, $\mu_1$ and $\mu_2$.

Calculate methanol removal for nitrate and nitrite using yield.

Calculate nitrate removal and change in nitrite from methanol removals.

Calculate new nitrate, nitrite and methanol concentrations at end of step.

Completed 100 steps?

Yes

Plot concentrations versus time.

Figure 5 - Mathematical model

The objective of running the model was to establish those conditions which would achieve an acceptable nitrite concentration (nominally <0.5mg/l) leaving the bed.
The inlet nitrate concentration was kept constant at 15mg NO₃ N/l, and the inlet nitrite concentration was set at zero. The stoichiometric methanol dose for this nitrate inlet concentration is 37mg/l, and the effects of underdosing (30mg/l) and overdosing (40mg/l) were compared.

Initially the model did not allow the $\mu_{\text{max}}$ values or proportion of "denitrifying" bacteria (those capable of reducing nitrate to nitrogen without releasing nitrite) to be varied at different levels in the bed, i.e. the values were kept constant throughout the depth of the bed. The model was later developed further to allow these variations within the bed to be investigated.

The model was used to investigate the effects of the following on nitrite production:

- changes in the values of $\mu_{1\text{max}}$ and $\mu_{2\text{max}}$ throughout the bed and variations within the bed,

- overdosing and underdosing of methanol,

- changes in biomass concentration within the bed,

- different proportions of denitrifying bacteria throughout the bed and variations within the bed.

4.4.2 Results

The shape of the nitrite profile is governed by the relative rates of nitrite production and removal; the former is a function of the maximum specific growth rate on nitrate ($\mu_{1\text{max}}$), and the latter a function of the maximum specific growth rate on nitrite ($\mu_{2\text{max}}$). The ratio of these two rates influences the size of the nitrite peak concentration. Increasing $\mu_{1\text{max}}$ will reduce the time taken to achieve a required degree of nitrate removal, but provided $\mu_{2\text{max}}$ is increased by the same proportion, the maximum nitrite would not be affected.
Results were obtained using a $\mu_{\text{max}}$ value of 0.6 and $\mu_{\text{max}}$ values of 1.2, 1.8 and 2.4 (i.e. a $\mu_{\text{max}} : \mu_{\text{max}}$ ratio of 2:1, 3:1 and 4:1).

The main conclusions to be drawn from running the original model with constant $\mu_{\text{max}} : \mu_{\text{max}}$ ratios (of up to 3:1) and proportions of denitrifying bacteria throughout the depth of the bed are:

(i) In the absence of denitrifying bacteria (capable of converting nitrate to nitrogen without releasing nitrite), high nitrite was produced under all conditions when underdosing of methanol occurred. The results suggest that a ratio of $\mu_{\text{max}}$ to $\mu_{\text{max}}$ of more than 3:1 is needed to reduce nitrite to acceptable levels. Changes in total biomass concentrations appear to have little effect on the shape of the nitrite profile (assuming equal concentrations of bacterial types capable of reducing nitrate to nitrite and nitrite to nitrogen).

(ii) At a $\mu_{\text{max}} : \mu_{\text{max}}$ ratio of 2:1 it is necessary to have 50% or more of the bacteria capable of complete nitrate reduction (without release of nitrite) to achieve acceptable nitrite. At the 3:1 ratio, a 25% proportion is satisfactory.

(iii) As observed in plant operation, overdosing of methanol results in acceptable nitrite concentrations leaving the bed, despite high concentrations within the bed, as a result of removal of nitrite when nitrate concentrations become low.

The original model did not allow either the $\mu_{\text{max}} : \mu_{\text{max}}$ ratio or the bacterial proportions to be varied over the depth of the bed. The model was then further developed to investigate the effects of varying those parameters at different depths within the fluidised bed. Results given in Appendix D indicate the effects of:

- varying $\mu_{\text{max}}$ between 1.2 and 2.4 (with $\mu_{\text{max}}$ at 0.6) at different depths in the bed,
- varying the proportion of denitrifying bacteria (nitrate to nitrogen without nitrite release) between zero and 75% at different depths in the bed with \( \mu_{z_{\text{max}}} : \mu_{1_{\text{max}}} \) kept constant at 1.2 : 0.6 (2:1).

Other conditions are described in Appendix D.

Table 5a summarises the results obtained from running the model with underdosing of carbon source and with no bacteria capable of complete reduction of nitrate to nitrogen without releasing nitrite. The results are for bacterial concentrations of 5, 10, 15 and 20g/l from the bottom of the bed to the top, because this is typical of the profile found from experience of operational plants. It would appear from the results on Table 5a that operation with a constant \( \mu_{z_{\text{max}}} : \mu_{1_{\text{max}}} \) ratio throughout the bed or with an increasing ratio from the bottom to the top of the bed give similar final nitrite concentration, whereas a decreasing ratio from bottom to top gives a much higher final nitrite for similar average \( \mu_{z_{\text{max}}} : \mu_{1_{\text{max}}} \) ratios.

Table 5b summarises the results of running the model with a varying proportion of denitrifying bacteria. At a \( \mu_{z_{\text{max}}} : \mu_{1_{\text{max}}} \) ratio of 2:1 with constant proportions of denitrifying bacteria throughout the bed, an average proportion of less than 50% of denitrifying bacteria gave unacceptable final nitrite concentration, irrespective of the distribution of the proportion through the bed. However, with a decreasing proportion of denitrifying bacteria from the bottom to top of the bed, final nitrite concentrations were higher than those for an increasing or constant proportion, and unacceptable nitrite was produced with an average proportion of 56%.

Generally, the results from the mathematical model suggest that to minimise nitrite concentrations, the proportions of nitrite reducing bacteria and denitrifying bacteria should be constant over the bed depth, or higher in the top half of the bed rather than the bottom half. Whilst the model does not yield any information of direct practical application, it has been a useful tool to investigate the fundamental biochemical factors which influence nitrite production, and add to an overall understanding of the process.
Table 5 - Summary of results from mathematical model

Table 5a - Effect of $u_{\text{max}} : u_{\text{lim}}$ ratio in the absence of bacteria capable of complete nitrate reduction without release of nitrite

<table>
<thead>
<tr>
<th>AVERAGE $u_{\text{max}} : u_{\text{lim}}$</th>
<th>DISTRIBUTION OF $u_{\text{max}} : u_{\text{lim}}$ IN BED (BOTTOM TO TOP)</th>
<th>FINAL NO₂N (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>Same throughout bed depth</td>
<td>2.5</td>
</tr>
<tr>
<td>3.0</td>
<td>Same throughout bed depth</td>
<td>0.8</td>
</tr>
<tr>
<td>4.0</td>
<td>Same throughout bed depth</td>
<td>0.6</td>
</tr>
<tr>
<td>3.7</td>
<td>3, 4, 4, 4</td>
<td>0.6</td>
</tr>
<tr>
<td>3.3</td>
<td>2, 3, 4, 4</td>
<td>0.6</td>
</tr>
<tr>
<td>3.0</td>
<td>2, 3, 3, 4</td>
<td>1.0</td>
</tr>
<tr>
<td>3.5</td>
<td>4, 4, 3, 3</td>
<td>1.1</td>
</tr>
<tr>
<td>2.7</td>
<td>4, 3, 2, 2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Notes: (i) Biomass concentrations 5, 10, 15, 20mg/l from bottom to top of bed.  
(ii) Feed concentrations: NO₃N = 15mg/l  
Methanol = 30mg/l

Table 5b - Effect of varying proportions of denitrifying bacteria with a constant $u_{\text{max}} : u_{\text{lim}}$ ratio of 2:1

<table>
<thead>
<tr>
<th>AVERAGE PROPORTION (%)</th>
<th>DISTRIBUTION IN BED (BOTTOM TO TOP)</th>
<th>FINAL NO₂N (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Same throughout bed depth</td>
<td>1.2</td>
</tr>
<tr>
<td>50</td>
<td>Same throughout bed depth</td>
<td>0.5</td>
</tr>
<tr>
<td>75</td>
<td>Same throughout bed depth</td>
<td>0.2</td>
</tr>
<tr>
<td>25</td>
<td>0, 25, 25, 50</td>
<td>1.5</td>
</tr>
<tr>
<td>37</td>
<td>0, 25, 50, 75</td>
<td>0.7</td>
</tr>
<tr>
<td>37</td>
<td>0, 50, 50, 50</td>
<td>0.7</td>
</tr>
<tr>
<td>50</td>
<td>25, 50, 50, 50</td>
<td>0.6</td>
</tr>
<tr>
<td>56</td>
<td>25, 50, 75, 75</td>
<td>0.3</td>
</tr>
<tr>
<td>62</td>
<td>25, 75, 75, 75</td>
<td>0.2</td>
</tr>
<tr>
<td>56</td>
<td>75, 50, 50, 50</td>
<td>0.7</td>
</tr>
<tr>
<td>44</td>
<td>75, 50, 25, 25</td>
<td>0.7</td>
</tr>
<tr>
<td>37</td>
<td>75, 50, 25, 0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Biomass and feed concentrations as on Table 5a.
SECTION 5 - REVIEW OF BIOLOGICAL DENITRIFICATION PROCESSES

5.1 PROCESS TYPES

5.1.1 General Considerations

Two basic types of biological denitrification process exist, using either heterotrophic or autotrophic bacteria. Heterotrophic processes are the more common, using bacteria which require an organic carbon source to be added for bacterial growth and metabolism. Methanol, ethanol or acetic acid are normally used as the carbon source. Autotrophic bacteria obtain their carbon from CO₂ in the water, and their energy from oxidation of inorganic materials such as sulphur or hydrogen. The proposed advantage of autotrophic processes lies in the fact that biodegradable organic compounds do not need to be dosed to the water. However, disadvantages include increased sulphate in the treated water or the need for larger plants because of the lower denitrification rates (see Section 5.2.).

In addition to these two basic process types, plants vary in the nature of the biomass support material, mode of operation and post-denitrification treatment.

Some of the more significant developments in biological denitrification in different countries are summarised below. Where possible, reference is made to implications on nitrite and carbon source residual control.

5.1.2 France

Two principal types of heterotrophic process have been developed in France:

"Biodenit" process (OTV)
"Nitrazur" process (Degremont)

Use of these processes was authorised by the French Ministry of Health in 1981 and the first plants began operation in 1983.
There are currently four plants operating in France, all treating groundwater sources, with a further two plants under design\(^9\). The four full-scale plants in operation are described in Table 6.

**Table 6 - Biological denitrification plants in France**

<table>
<thead>
<tr>
<th>SITE</th>
<th>MANUFACTURER</th>
<th>CARBON SOURCE</th>
<th>YEAR COMMISSIONED</th>
<th>FLOW RATE (m(^3)/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Champfleur</td>
<td>Degremont</td>
<td>Ethanol</td>
<td>1984</td>
<td>70</td>
</tr>
<tr>
<td>Chateau Landon</td>
<td>Degremont</td>
<td>Acetic acid</td>
<td>1983</td>
<td>50</td>
</tr>
<tr>
<td>Eragny</td>
<td>OTV</td>
<td>Ethanol</td>
<td>1983</td>
<td>80</td>
</tr>
<tr>
<td>Guernes</td>
<td>OTV</td>
<td>Ethanol</td>
<td>1987</td>
<td>400</td>
</tr>
</tbody>
</table>

The Degremont design uses an upflow fixed bed of a proprietary granular material ("Biolite L") of effective size 1.7 or 2.7mm as the denitrification reactor, followed by a granular activated carbon (GAC) filter. The OTV design uses a downflow denitrification reactor, also with proprietary biomass support material ("Biodagene", 3-6mm), followed by a dual media aerated filter of the Biocarbonate design.

The plants are operated to achieve only partial denitrification, and it is claimed that control of residual nitrite can be maintained by frequent cleaning of the bed to remove excess biomass. However, the Degremont process can produce excessive nitrite if it is subject to frequent interruptions to operation. At such times it is reported that excess nitrite can be removed in the subsequent GAC filters, presumably by biological action (nitrification) although no information is given on the mechanism of removal. The Biocarbonate filter in the OTV process is likely to be effective in maintaining low carbon source and nitrite residuals by biological activity (conversion of nitrite to nitrate by nitrifying bacteria, and carbon source removal by aerobic bacteria).
Continuous monitoring of ethanol or acetic acid is not carried out, although it is reported that carbon source has never been detected in the final water, presumably based on routine spot sampling and analysis.

Another heterotrophic process has been developed to pilot scale in France, using an upflow bed of sand or other granular material with continuous removal of excess biomass\(^{10}\). Results are given in which complete denitrification is reported, during which time nitrite residuals are at non-detectable levels. However, results for nitrite during later operation with partial denitrification are not given.

A heterotrophic process providing denitrification in-situ within the aquifer has been investigated, using beds of straw to provide the carbon source\(^{11}\). Water is pumped from a central well, and part of the flow is passed to surrounding beds of straw. Organic material is leached from the bed (and some denitrification occurs, evident from increased nitrite) and the water is re-infiltrated into the aquifer, where further denitrification occurs using the organic material leached from the straw. No problems of nitrite or carbon source in the final treated water taken from the central well are reported.

Very little work on autotrophic denitrification has been reported from France. Laboratory scale investigations using packed beds of iron pyrite (FeS\(_2\)) have been carried out\(^{12}\), in which denitrification is due partly to biological activity of autotrophic bacteria (Thiobacillus denitrificans and Ferrobacillus ferroxidans, which oxidise sulphur and iron respectively) and partly to chemical reduction of nitrate. Some work on denitrification using sulphur/limestone beds has also been reported\(^{13,14}\), based on Thiobacillus denitrificans bacteria. The mechanism is described in more detail in the work reported from the Netherlands (Section 5.1.4).

5.1.3 Germany

A heterotrophic process was developed at Neuss involving a low flow bed of granular activated carbon (GAC) of particle size 2-4mm as a biomass support, with acetic acid as the carbon source\(^{15,16}\). Pilot plant
trials treating groundwater at 10m³/h indicated occasionally high nitrite in the treated water, up to 0.5mg NO₂/l (0.15mg N/l) but control to less than 0.1mg NO₂/l was possible by selection of suitable (unspecified) backwash conditions for cleaning of the bed. The treated water was aerated and reinfiltirated into the aquifer to give roughly 50 days retention, during which nitrite was removed by nitrification and excess acetic acid removed by aerobic bacterial activity. Acetic acid was not measured directly, but dissolved organic carbon (DOC) was used as an indication of the residual carbon source concentration. Reported values for DOC in the denitrified water and the water 10m downstream from the injection well (roughly 10 hours retention) are 1.7mg/l and 0.9mg/l respectively, indicating the potential for removal of excess carbon source underground. The plant was operated with underdosing of carbon source (carbon source limited) to have a small nitrate residual of 5mg NO₃/l (1.1mg NO₃N/l) in the treated water. Any residual carbon source would therefore result from biomass limitation (i.e. insufficient biomass in the bed to achieve full denitrification). A full scale plant was under construction in 1989, to treat 7ml/d by denitrification, multi-media filtration, reaeration and infiltration.

Also at Neuss, in-situ heterotrophic denitrification was investigated by injection of acetic acid into the aquifer[16]. Severe problems were encountered with dissolution of manganese and blockage by bacteria in the anoxic zone developed for denitrification. Problems with blockage were reduced by intermittent dosing of high acetic acid concentrations to reduce the pH. Manganese problems were overcome by reaerating the water and causing precipitation of the manganese in an aerobic region downstream of the anoxic zone. However, this type of in-situ treatment was not considered to be feasible because of these problems and the large areas of land needed; roughly 2 ha were used to treat 30m³/hour, with a considerable number of boreholes for monitoring and control purposes.

An upflow fixed-bed heterotrophic process using polystyrene beads as a biomass support has been developed by Preussag, (the "Denipor" process), and investigated at full-scale treating a small proportion of an 800m³/h source[14]. The process achieves 95% denitrification with a feed water nitrate of 60mg NO₃/l (13.6mg NO₃ N/l).
Nitrite production could be controlled by overdosing of ethanol, with excess ethanol being removed by two-stage filtration under aerobic conditions.

A heterotrophic process based on rotating biological contactor (RBC) package plants is being marketed by Norddeutsche Seekabelwerke (NSW), aimed primarily at treatment of small groundwater sources\(^{17}\). The process uses two stages of RBC (anoxic and aerobic) followed by sand/GAC filtration.

Complete denitrification can be achieved in the anoxic stage, followed by removal of excess carbon source (ethanol) in the aerobic RBC. No nitrite problems are reported\(^{18}\).

A full-scale autotrophic process is in operation near Monchengladbach, using hydrogen as the energy source and a fixed bed of plastic media as the biomass support\(^{19,20}\). The process (Denitropur) was developed by the Sulzer company, and the plant treats a groundwater source at 50m\(^3\)/hour in up to 9 denitrification reactors in series, followed by dual-media filtration. Nitrate removal from 80mg NO\(_3\)/l (18.2mg NO\(_3\)N/l) to less than 5mg NO\(_3\)/l (1.1mg NO\(_3\)N/l) is achieved. High nitrite was encountered during the start-up period, but was subsequently maintained at less than 0.1mg NO\(_2\)/l, perhaps as a result of the high level of denitrification (low treated water nitrate) achieved. Excess hydrogen can be removed from the denitrified water by aeration before filtration. Carbon dioxide is used to control the pH between each denitrification reactor. The denitrification reaction is:

\[
5 \text{H}_2 + 2 \text{H}^+ + 2 \text{NO}_3^- \rightarrow \text{N}_2 + 6 \text{H}_2\text{O}
\]

To achieve adequate hydrogen concentrations in solution the plant is operated at a pressure of 4 to 6 atmospheres.
5.1.4 Netherlands

An autotrophic process has been developed to large pilot scale (35m³/hour) for denitrification of groundwater using a bed of mixed sulphur and limestone granules. The process relies on growth of Thiobacillus denitrificans bacteria on the sulphur granules, which can bring about nitrate reduction by oxidation of the sulphur:

\[ 5 \text{S} + 6 \text{NO}_3^- + 2 \text{H}_2\text{O} \rightarrow 3 \text{N}_2 + 5 \text{SO}_4^{2-} + 4 \text{H}^+ \]

The limestone in the bed helps maintain a suitable pH for denitrification (pH 6.4 - 6.8) and provides a source of carbon for bacterial growth. The feed water to the process needs to be vacuum degassed to remove nitrogen and oxygen to prevent blockage of the bed by nitrogen bubbles produced by denitrification. The denitrified water is re-infiltrated into the aquifer. Completion denitrification is achieved, and no problems with high nitrite were reported. However, the treated water contains significantly increased concentrations of readily biodegradable material (measured as assimilable organic carbon, AOC), which may need removal to prevent problems of bacterial growth in distribution. This was reduced to a satisfactory level by infiltration, although potential problems with blockage of the infiltration basin were envisaged.

The use of heterotrophic biological treatment for denitrification of ion exchange waste regenerant has been investigated at pilot scale in the Netherlands. This approach offers benefits with respect to reduction in chemical usage, the volume of waste for disposal, and an improvement in the quality of the waste to reduce its impact on the environment. Such an approach would only offer cost benefits for situation where ion exchange waste needed to be transported by tanker over long distances. However, future restrictions on the disposal of high nitrate waste will make this combined process more viable, and further development is justified. Nitrite production and carbon source residuals are less important for this process, because the biological denitrification stage does not come directly into contact with the treated water.
Pilot plant trials with fixed bed heterotrophic denitrification are also being carried out, with particular emphasis on the post-denitrification treatment needed to produce suitable quality water for distribution, and on ethanol monitoring and control(25).

5.1.5 Belgium

A heterotrophic biological fluidised bed process has been investigated at pilot scale at the Blankaart works serving Brussels, using methanol as the carbon source. A full scale plant is planned for this site. The pilot plant included post-denitrification aerobic biological treatment in a gravel filter for removal of excess methanol. Investigations included the dosing of excess methanol to achieve high levels of denitrification. The reported results of the work do not mention nitrite concentrations in the treated water(26).

Autotrophic denitrification has been investigated at laboratory scale using hydrogen as the energy source, with a dowflow fixed bed of bacteria supported on a polyurethane foam matrix(27). Complete denitrification is not achieved, and up to 5mg NO₂/1 leaving the denitrification reactor is removed by subsequent aerobic treatment for nitrification (NO₂ to NO₃).

5.1.6 Sweden

An in-situ denitrification system ("Nitredox") has been developed and a number of plants installed in Sweden and other countries(28).

The process involves two concentric rings of injection wells around a central supply well. The outer ring injects carbon source (methanol) and the inner ring injects aerated water. Denitrification occurs in the anoxic region between the outer and inner ring, and the aerobic region between the inner ring and the supply well provides for nitrite removal (nitrification), excess methanol removal, and removal of iron and manganese if necessary. The system described is more compact than that operated in Germany (Section 5.1.3), with the outer ring being only 18m radius for a supply well output of 60 l/s.
5.1.7 Summary of the Process Review

The occurrence and significance of nitrite is mentioned in almost all of the reported work. In some cases control of the concentration leaving the denitrification stage is reported to be achieved by establishing suitable operating conditions with respect to cleaning of the bed to remove excess biomass. Regular cleaning to remove "older" biomass is claimed to prevent nitrite formation, although frequent shut-down and restart (which occurs during cleaning for some processes) can induce nitrite production. This would appear to give distinct advantages to the BFB process, which is essentially continuous and does not need to be shut-down for sand cleaning. However, experience has shown that this process is also prone to problems of nitrite.

Some processes achieve high levels of denitrification, and nitrite may not be a problem because of this. However, deliberate overdosing of carbon source to control nitrite by achieving complete denitrification is mentioned for only one of the reported processes. Virtually all of the processes have some form of post-denitrification biological treatment, but this seems to be included to achieve nitrification for nitrite removal and to remove excess carbon source resulting from biomass limitation in the denitrification reactor. The latter is likely to occur in systems which are cleaned by backwashing, which does not allow good control of biomass removal. Another advantage of the BFB process is that sand cleaning can be controlled very accurately to prevent removal of too much biomass; hence the BFB system need never become biomass limited provided it is operated within design conditions.

The use of nitrification to remove residual nitrite is preferable to using chlorination, in that high chlorine doses, which could lead to trihalomethane production, are not required. However, nitrification can be unstable and is strongly temperature dependant. Also, there is inherent inefficiency in allowing nitrite to be produced from nitrate, and then converting this nitrite back to nitrate again. This could be acceptable with low levels of nitrite, but higher levels will start to make the process inefficient with regard to overall nitrate removal.
Prevention of high nitrate concentrations in the water leaving the denitrification reactor must therefore be preferable to its subsequent removal either chemically (using chlorine) or biologically by nitrifying bacteria.

Performance of in-situ denitrification systems has been inconsistent. Whilst success has been reported for work carried out in Sweden\textsuperscript{(28)}, Israel\textsuperscript{(29)} and Czechoslovakia\textsuperscript{(30)}, severe problems of aquifer blockage were reported from Germany\textsuperscript{(16)} and the Netherlands\textsuperscript{(31)}. It is likely that the nature of the aquifer, as well as operating conditions, will have a major influence of the success of in-situ denitrification.

5.2 GUIDELINES FOR PROCESS SELECTION, DESIGN AND OPERATION

5.2.1 Process Selection

In situ denitrification offers potential advantages with regard to capital costs because plant and equipment requirements are minimal. However, the area of land required may be substantial, depending upon the properties of the aquifer and the level of denitrification required. The risks associated with failure of in situ systems are much greater than those for treatment plant, in that it may be necessary to abandon boreholes because of aquifer blockage. Treatment plants are therefore likely to be the preferred option for most circumstances.

Autotrophic denitrification using hydrogen by the Denitropur process can allow overdosing of energy source for complete denitrification (to avoid nitrite production), with relatively easy removal of the excess hydrogen by air stripping. However, plant and equipment requirements are substantial compared with heterotrophic process because:

- the process needs to operate at high pressure (4-6 atmospheres) to ensure adequate dissolution of hydrogen,
- denitrification rates are low, and the biological reactors need to be at least four times the volume of heterotrophic reactors for the same duty (giving 1 to 2 hours retention compared with 15 to 30 minutes).
These plant requirements give a relatively high capital cost for the process. The plant at Monchengladbach, treating 50m³/h to remove 70-80mg NO₃/l, had a capital cost of approximately £400,000²⁰, (1985) which is roughly double the cost of a biological fluidised bed plant of equivalent duty³². The operating costs are reported as roughly 10p/m³ treated, which is also high relative to those for BFB denitrification using methanol, ethanol or acetic acid. Probably about half of these operating costs will be for hydrogen. The hydrogen requirements are 1g H₂ : 10g NO₃, and a plant treating 1ML/d to remove 70mg NO₃/l (15.9mg NO₂N/l) would therefore require 7kg H₂ per day. UK costs for hydrogen are roughly £50 per 100m³ at NTP, or approximately £6 per kg. The hydrogen cost for treating 1ML/d is therefore £42 per day or 4.2p/m³ treated.

Storage of sufficient hydrogen to last 1 week to treat 1ML/d would require a storage volume of about 15m³, assuming storage under a pressure of 40 atmospheres¹⁹. Storage and handling requirements for such quantities of hydrogen need due consideration of the appropriate regulations. The process is suitable for treatment of raw surface waters as well as groundwater.

The sulphur/limestone process also allows complete denitrification without problems of excess energy source in the treated water. However, the increase in sulphate needs to be considered. Each 100mg NO₃/l (22.6mg NO₂N/l) removed adds 170mg SO₄/l²¹. The sulphate increases to achieve 40mg NO₃/l (9.1mg NO₂N/l) in supply, for a range of raw water nitrate concentrations, are shown in Table 7.

Table 7 - Sulphate increase in sulphur/limestone denitrification

<table>
<thead>
<tr>
<th>RAW WATER (mg NO₃/l)</th>
<th>INCREASE IN SO₄ (mg/l)</th>
<th>MAXIMUM RAW WATER SULPHATE (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>51</td>
<td>199</td>
</tr>
<tr>
<td>90</td>
<td>85</td>
<td>165</td>
</tr>
<tr>
<td>110</td>
<td>119</td>
<td>131</td>
</tr>
</tbody>
</table>
The third column in the above table shows the maximum sulphate that can be accepted in the raw water to avoid exceeding the Water Quality Regulations prescribed concentration of 250mg SO₄/1. On this basis the process is unlikely to be suitable for treating water from limestone aquifers, but may be suitable for the lower sulphate waters from chalk or sandstone aquifers. The area of land required also needs to be considered. The process operates at a low filtration rate of about 0.25m/h. Each 1ML/d treated therefore requires an area of 167m², for example with dimensions of 20m x 8.3m. The process offers simplicity of operation however, making it suitable for remote sites. The bed is operated continuously until nitrate breakthrough occurs. The bed is then dug out and recharged with fresh sulphur/limestone granules. A 1.5m deep bed should operate for over one year removing 75mg NO₃/1(21). The process has been demonstrated for groundwater treatment, but not for surface waters. Raw surface waters containing high turbidity would be unsuitable because of the potential for blockage of the bed.

The range of heterotrophic processes developed differ with respect to carbon source and biomass support material. However, choice of carbon source is primarily political rather than technical, and each of the process types is likely to operate satisfactorily with either methanol, ethanol or acetic acid as the carbon source. In the UK, methanol is the cheapest carbon source, and the use of ethanol or acetic acid would increase chemical costs by factors of 2 or 3 respectively.

The fixed bed processes use relatively coarse media for biomass support, and therefore would be suitable for treatment of raw surface water as well as groundwater. However, surface waters with high turbidity or suspended solids concentrations may cause rapid blockage of the bed, and consequently high backwash frequency. The biological fluidised bed (BFB) process does not suffer from problems of blockage, and can handle waters with very high turbidity without deterioration in performance. Other advantages of the BFB process over fixed bed processes are:
- the ability to control biomass removal, and to remove excess biomass without stopping operation,
- the use of media with small particle size, giving high biomass concentrations per unit volume of reactor.

However, the latter is not likely to be significant enough to have a major influence of capital costs for the plants.

5.2.2 Design and Operation of BFB Plants

(i) Denitrification plant design

Details of BFB denitrification plant design and operation are given in a WRC report\(^{(32)}\).

The process uses fine sand (0.3 - 0.5mm) as a biomass support, in beds fluidised by upflow of the treated water at rates of 20m/h. Removal of dissolved oxygen (DO) occurs at the bottom of the bed, establishing anoxic conditions for nitrate removal in the higher regions of the bed.

The depth of fluidised bed required is a function of the dissolved oxygen and nitrate concentrations in the feed water, the average biomass concentration in the bed, and the rates of DO and nitrate removal at the minimum operating temperature. Design figures that can be used are as follows:

Average biomass (volatile solids) concentration = 15kg VS/m\(^3\) bed

Removal rates at 2°C using methanol:

\[
\begin{align*}
\text{DO removal} & = 18\text{g DO/(kg VS.h)} \\
\text{Nitrate removal} & = 5.5\text{g NO}_3\text{N/(kg VS/h)}
\end{align*}
\]

Using these figures, the required fluidised bed depth can be calculated from the raw water DO and nitrate concentrations, the total depth required being the sum of the requirements for DO and nitrate.
The requirement for DO removal is 0.75m depth per 10mg DO/l feed water. The requirement for nitrate removal is 0.24m per mg NO₃N/l removed.

The plant should be designed to give sufficient bed depth to allow complete denitrification (i.e. the design denitrification capacity should be based on the maximum feed water nitrate concentration).

The plant would be used to treat only a proportion of the total output to achieve the desired overall nitrate removal. The fraction \( F \) of the total flow treated is calculated from:

\[
F = \frac{N_0 - N_D}{N_R}
\]

where
\( N_0 \) = raw water nitrate concentration
\( N_D \) = nitrate concentration in distributed water
\( N_R \) = nitrate removed by treatment
\( (N_R = N_0 \) for complete denitrification)

Carbon source requirements for dissolved oxygen and nitrate removal are shown in Table 8.

**Table 8 - Carbon source requirements**

<table>
<thead>
<tr>
<th></th>
<th>FOR DO REMOVAL (mg/mg DO)</th>
<th>FOR NITRATE REMOVAL (mg/mg NO₃N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>1.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>
(ii) Post-denitrification treatment

Pilot plant trials described in this report have indicated that the process should be operated to achieve complete denitrification, and to leave a small residual carbon source concentration in the denitrified water, to be removed by subsequent treatment. The latter would consist of aeration followed by, for example, floc blanket clarification and/or rapid gravity filtration. It would be more efficient to operate continuously with a small overdose of carbon source, rather than overdose only when nitrite problems occur, because the post-denitrification treatment will need time to develop biological activity. This may take a few days, and the excess carbon source resulting from a sudden increase in dose to prevent nitrite would not be removed effectively.

For most works, less than 50% of the total flow would need to be denitrified, and then blended in with rest of the flow before distribution. It would be better to treat the denitrified flow separate to the non-denitrified flow, rather than mix the two before post-denitrification treatment, for the following reasons:

- biological removal of excess carbon source is likely to be more efficient with higher starting concentrations,
- problems with denitrification or subsequent biological treatment will be restricted to a proportion of the total flow, leaving the non-denitrified flow to be blended into supply if necessary,
- modifications can be carried out to the post-denitrification treatment units to enhance biological activity, perhaps, for example, by replacing the existing single media filtration with dual or multi-media beds.

Most surface water treatment works have a number of parallel treatment streams, and it should be relatively easy to modify the works to handle the denitrified flow separate to the remainder.
It may also be relatively easy to provide a cross-connection between the denitrified and non-denitrified flow such that mixing could be achieved before post-denitrification treatment. This flexibility of operation could be beneficial in some circumstances. For example, if problems with the post-denitrification treatment occurred, the denitrification plant could be operated under carbon limited condition (i.e. with underdosing of carbon source) and the denitrified flow mixed with the non-denitrified flow for treatment while the problems with the normal post-denitrification treatment were solved.

At surface water works with floc blanket clarification and rapid gravity filtration, the existing treatment should give effective removal of excess carbon source without modification. Works with clarification by dissolved air flotation (DAF) would be less efficient because of the short retention in the DAF unit, and because of removal of biomass carry-over by DAF (carry-over of biomass from the BFB unit may be a significant factor in the rapid development of biological activity in floc blanket clarifiers or rapid gravity filters).

If two-stage filtration is carried out after DAF, the retention time in the filters may be long enough to ensure adequate removal of carbon source. If the works has single-stage filtration after DAF, it might be necessary to install an additional filtration stage after denitrification but before chemical coagulation. This additional filtration stage could be operated at relatively high rate (10m/h) using coarse media (e.g. 8/16 mesh sand). Similarly, at works with chemical coagulation and single-stage direct filtration without clarification, an additional high rate filtration stage before chemical coagulation would be beneficial. It may be possible at some works to use existing filter shells to provide the additional filtration stage.

No pilot plant trials have been carried out on groundwater to investigate the extent of treatment needed to restore the quality after denitrification. It is likely that rapid gravity sand
filtration without chemical coagulation would be enough to remove bacterial floc carried over from the denitrification reactor, and also to remove excess carbon source. A relatively low filtration rate (say 5m/h) would be recommended, using 8/16 mesh sand. Pilot plant trials would be needed to confirm this approach for groundwater, and are also recommended prior to installation of BFB denitrification at surface water treatment works.

(iii) Process monitoring and control

The degree of overdosing that is acceptable (i.e. that can be removed by subsequent treatment) depends on the carbon source used and the extent of reaeration of the denitrified water that can be achieved. Dissolved oxygen saturation varies with water temperature between 9mg/l (20°C) and 15mg/l (5°C). The DO requirements for methanol, ethanol and acetic acid are shown on Table 8. Assuming 10mg DO/l can be achieved, the maximum carbon removal possible to maintain aerobic conditions in the post-denitrification treatment would be roughly 8mg/l for methanol and acetic acid, and 4mg/l for ethanol. To ensure a margin of safety, the recommended maximum overdose would be 5mg/l for methanol and acetic acid, and 3mg/l for ethanol.

Monitoring and control of the process could be based on a feed-forward control system, in which the carbon source dose is controlled automatically based on monitoring of the feed water DO and nitrate concentrations. However, this system on its own may give unsatisfactory results, because small errors in measurement can give large differences in carbon source dose (and, therefore, residual after denitrification). For example combined errors of ±1mg/l for DO and NO₃-N would give ±3.5mg/l for methanol, which would probably be unacceptable for control purposes. It would therefore be better to combine feed-forward control with a feed-back loop based on carbon source monitoring, which would override the feed-forward control if necessary. The monitoring regime would ideally operate as follows:
- the feed water DO and nitrate would establish the required carbon source dose to leave a small carbon source residual after denitrification (say 1 or 2mg/l),

- carbon source monitoring after denitrification would provide a control signal to trim the carbon source dose as necessary to maintain the residual between desired limits,

- carbon source monitoring after post-denitrification treatment would be used to initiate an alarm and reduce (or turn-off) the carbon source dose if the residual concentration rose above a pre-set level,

- monitoring of nitrate and nitrite in the denitrified water, which would normally be at low or non-detectable levels, would be used to initiate an alarm as an indicator of potential failure of the process as a result of inadequate dosing of carbon source or loss of biological activity of the bed. (This is essentially a back-up to carbon source monitoring at this point.)

This monitoring regime is illustrated in Figure 6.

Simpler monitoring and control regimes are possible, and may be adequate for some circumstances. For example for waters with relatively constant nitrate and DO concentrations, the dose could be set manually, and monitoring of carbon source residual after post-denitrification treatment used to trim the dose to maintain the residual below a pre-set level. This would also require nitrate and nitrite monitoring of the denitrified water to guard against failure of carbon source dosing or loss of activity of the bed.
Figure 6 - Monitoring and control regime
(1) Biosensors offer potential for development of monitoring systems for methanol, ethanol or acetic acid. Such systems, once developed, should be relatively inexpensive to install and operate. However, development costs for a prototype system would be approximately £50,000.

(2) The VOC monitor being developed by WRc Medmenham is capable of giving detection limits of less than 1mg/l for methanol and ethanol, but gave a poor response for acetic acid. In the further development of this system, consideration should be given to its potential use for monitoring of biological denitrification carbon source residuals. It is likely to offer significant cost advantages over alternative techniques.

(3) Gas chromatography using head-space vapour injection has been demonstrated to be sensitive enough for monitoring of methanol and ethanol residuals down to 1mg/l or less, but not for acetic acid. Proprietary continuous monitoring systems based on this technique are available, and should be suitable for monitoring and control of biological denitrification processes.

(4) Pilot plant trials have indicated that control of nitrite residuals can be achieved by deliberately overdosing carbon source, to operate under nitrate limited conditions. Excess methanol of up to 5mg/l could be reduced to non-detectable levels by the development of biological activity during floc blanket clarification and/or rapid gravity filtration, the latter with or without chemical coagulation.

(5) Based on these pilot plant results, future biological fluidised bed denitrification plants should be designed for nitrate limited operation, and subsequent conventional treatment operated to establish biological activity for removal of excess carbon source. This may require some modification to existing plant.
A mathematical model of biological fluidised bed denitrification has been developed and used to help understand some of the biochemical factors which lead to excess nitrite production under carbon limited conditions (i.e. with underdosing of carbon source to achieve only partial denitrification). The model investigated the effects of variations within the fluidised bed of the proportions of nitrate reducing bacteria, nitrite reducing bacteria, and denitrifying bacteria (those capable of reducing nitrate to nitrogen without releasing nitrite). The results indicate that there are no advantages, with respect to final nitrite concentrations, in having varying proportions of these bacteria compared with constant proportions throughout the bed depth. In fact, the results suggest that for similar average proportions in the bed, decreasing proportions of denitrifying or nitrite reducing bacteria from the bottom of the bed to the top can lead to higher final nitrite concentrations.

Analysis of bed profile data from operational plants and laboratory experimental batch tests did not provide any useful information regarding the factors influencing nitrite production.
REFERENCES


(3) MUSSELWHITE C C. An Automated Method for the Determination of Residual Methanol in Denitrified Effluents. Water Pollution Control, 74, 1975, No. 1, 110.


(17) BUCKLEY D S. A continuous biological process for nitrate elimination from potable water. European Water and Sewage, April 1986.

(18) BUCKLEY D S. NSW. Personal communication, March 1988.


(25) VAN DER HOEK J P. KIWA, Personal communication, March 1990.


APPENDIX A

PRINCIPLES OF ENZYME TEST KITS

FOR ETHANOL AND ACETIC ACID
APPENDIX A

PRINCIPLES OF ENZYME TEST KITS FOR ETHANOL AND ACETIC ACID

A.1 ETHANOL

Ethanol is oxidised in the presence of the enzyme alcohol dehydrogenase (ADH) by nicotinamide-adenine dinucleotide (NAD+) to produce acetaldehyde under alkaline conditions. The acetaldehyde is then oxidised to acetic acid in the presence of aldehyde dehydrogenase (ALDH).

\[
\text{ADH} \\
\text{Ethanol + NAD}^+ \rightarrow \text{Acetaldehyde + NADH + H}^+
\]

\[
\text{ALDH} \\
\text{Acetaldehyde + NAD}^+ \rightarrow \text{Acetic acid + NADH + H}^+
\]

The reduced NAD (NADH) produced is determined by absorbance at 334, 340 or 365nm.

A.2 ACETIC ACID

Acetate is converted to acetyl CoA in the presence of the enzyme acetyl-CoA synthetase (ACS), adenine-5-triphosphate (ATP) and co-enzyme A (CoA).

\[
\text{ACS} \\
\text{Acetate + ATP + CoA \rightarrow acetyl-CoA + AMP + pyrophosphate}
\]

Acetyl-CoA reacts with oxaloacetate to form citrate in the presence of citrate synthetase (CS).

\[
\text{CS} \\
\text{Acetyl-CoA + oxaloacetate + H}_2\text{O \rightarrow citrate + CoA}
\]

The oxaloacetate required for this reaction is formed from malate and NAD+ in the presence of the enzyme malate dehydrogenase (MDH).
MDH
Malate + NAD⁺ ----> oxaloacetate + NADH + H⁺

The amount of NADH produced is measured as for the ethanol test, and is related to the amount of acetic acid present in the sample.
APPENDIX B

INFORMATION ON ENZYME BASED MONITORING PROCEDURE
FROM BIRD ENGINEERING
APPENDIX B - INFORMATION ON ENZYME BASED MONITORING PROCEDURE
FROM BIRD ENGINEERING

The technique is based on a dual enzyme system with the following reactions:

\[
\begin{align*}
C_2H_5OH + O_2 \rightarrow & \text{CH}_3\text{CHO} + H_2O_2 \\
\text{alcohol oxidase} \\
H_2O_2 + \text{acceptor} \rightarrow & \text{H}_2\text{O} + \text{coloured acceptor} \\
\text{peroxidase}
\end{align*}
\]

The concentration of the coloured product is directly proportional to the alcohol concentration, and is measured using a spectrophotometer at a wavelength of 500m. Measurement can be made continuously using an on-line colorimeter. The manufacturer of the system claims that concentrations down to 0.5mg/l can be determined.

More information can be obtained from:

Dr G Mijnbeek
Bird Engineering BV
De Brauweg 13
3125 AE Schiedam
The Netherlands

Telephone: 010 415 7822
APPENDIX C

SUMMARY OF REPORT ON BIOSENSORS FROM CRANFIELD BIOTECHNOLOGY CENTRE
APPENDIX C – SUMMARY OF REPORT ON BIOSENSORS
FROM CRANFIELD BIOTECHNOLOGY CENTRE

An overview of biological fluidised bed denitrification technology in relation to carbon source monitoring is given, highlighting the specific requirements of a BFB denitrification carbon source monitor. Biosensor technology is briefly described and past literature concerned with the monitoring of methanol, ethanol and acetate using biocatalysts is reviewed.

Based on this review, biocatalysts and transducer systems which would be best suited to the analysis of methanol, ethanol or acetate in a BFB denitrification plant are recommended. For all three carbon sources, flow injection analysis with either amperometric or spectrophotometric detection is recommended, combined with either alcohol oxidase or alcohol oxidase/peroxidase/potassium hexacyanoferrate (II) as the biocatalyst system for methanol analysis, alcohol dehydrogenase/diaphorase/potassium hexacyanoferrate (III) as the biocatalyst system for ethanol analysis, and alcohol oxidase/peroxidase/potassium hexacyanoferrate (II) as the biocatalyst system for acetate analysis.

The cost of constructing a flow injection analysis system with amperometric detection was estimated at approximately 8,000 to 10,000 pounds for a manual flow injection analysis system and 9,500 to 14,000 pounds for an automatic flow injection analysis system. The cost of a complete flow injection analysis system with spectrophotometric detection was estimated at approximately 8,000 to 13,000 pounds for the manual flow injection analysis system and 9,000 to 16,000 pounds for the automatic flow injection analysis system. To construct a prototype system ready for evaluation by the WRC, it was estimated that approximately 12 months of skilled/technical labour would be required, with 2 to 3 months operational training of non-skilled personnel.

A list of contacts for further information is provided, plus all of the papers referred to in this report.
APPENDIX D

RESULTS FROM MATHEMATICAL MODEL
APPENDIX D
RESULTS FROM MATHEMATICAL MODEL

Conditions for all runs:

Feed $\text{NO}_3\text{N} = 15\text{mg/l}$
Feed $\text{NO}_2\text{N} = 0\text{mg/l}$
Feed methanol = 30mg/l

Half-rate constants (mg/l):

$k_{\text{NO}_3} = 0.7$
$k_{\text{NO}_2} = 1.0$
$k_m = 2.0$

Bacterial yield = 0.25g/g methanol

Biomass concentrations in bed (g/l)

Bottom = 5
1/3 up = 10
2/3 up = 15
Top = 20

Maximum specific growth rate for nitrate reduction ($\mu_{\text{amax}}$) = 0.6h$^{-1}$

The results from the model, using the conditions given above and on the following tables, are shown in graphical form on Figures A1 to A6, and B7 to B16. The graphs show predicted concentrations of nitrate, nitrite and methanol against time. The latter is equivalent to distance from the bottom of the fluidised bed, and therefore the concentrations represent profiles from the bottom of the bed (time = 0) to the top.

(i) Runs A1 to A6; proportion of denitrifying bacteria = 0. Varying $\mu_{\text{amax}}$ throughout bed. Predicted concentration profiles are shown in Figures A1 to A6.
<table>
<thead>
<tr>
<th>RUN</th>
<th>( \mu_{2\text{max}} ) IN BED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOTTOM</td>
</tr>
<tr>
<td>A1</td>
<td>2.4</td>
</tr>
<tr>
<td>A2</td>
<td>1.8</td>
</tr>
<tr>
<td>A3</td>
<td>1.2</td>
</tr>
<tr>
<td>A4</td>
<td>1.2</td>
</tr>
<tr>
<td>A5</td>
<td>2.4</td>
</tr>
<tr>
<td>A6</td>
<td>2.4</td>
</tr>
</tbody>
</table>

(ii) Runs B7 to B16; \( \mu_{2\text{max}} = 1.2:0.6 \) varying proportions of denitrifying bacteria. Predicted concentration profiles are shown in Figures B7 to B16.

<table>
<thead>
<tr>
<th>RUN</th>
<th>PROPORTION OF DENITRIFYING BACTERIA IN BED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOTTOM</td>
</tr>
<tr>
<td>B7</td>
<td>0.00</td>
</tr>
<tr>
<td>B6</td>
<td>0.00</td>
</tr>
<tr>
<td>B9</td>
<td>0.00</td>
</tr>
<tr>
<td>B10</td>
<td>0.25</td>
</tr>
<tr>
<td>B11</td>
<td>0.25</td>
</tr>
<tr>
<td>B12</td>
<td>0.25</td>
</tr>
<tr>
<td>B13</td>
<td>0.50</td>
</tr>
<tr>
<td>B14</td>
<td>0.75</td>
</tr>
<tr>
<td>B15</td>
<td>0.75</td>
</tr>
<tr>
<td>B16</td>
<td>0.75</td>
</tr>
</tbody>
</table>

- D/2 -
A1: BIOMODEL: PREDICTED CONC. PROFILES OF BFB

---

**Concentration (mg/L)**

- **Nitrate**
- **Nitrite x10**
- **Methanol**

**Time (mins)**

0 10 20 30 40 50
A2: BIOMODEL; PREDICTED CONC. PROFILES OF BFB

Nitrate

Nitrite x10

Methanol

Concentration (mg/l)

Time (mins)
A3: BIOMODEL; PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/l) vs. Time (mins)
A 4: BIOMODEL: PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/l)

Time (mins)
A5: BIOMODEL: PREDICTED CONC. PROFILES OF BFB

- --- Nitrate
- --- Nitrite x10
- --- Methanol

Concentration (mg/l)

Time (mins)
A6: BIOMODEL; PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/L)

Time (mins)
B7: BIOMODEL: PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/l)

0 5 10 15 20 25 30 35 40

Time (mins)

0 10 20 30 40 50
BB: BIOMODEL; PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol
B9: BIOMODEL: PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/L)

Time (mins)
B10: BIOMODEL: PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrile x10
- Methanol

Concentration (mg/L) vs. Time (mins)
Bi11: Biomodel; Predicted conc. profiles of BFB

- **Nitrate**
- **Nitrite x10**
- **Methanol**

**Concentration (mg/l)**

**Time (mins)**
B12: BIOMODEL: PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/l)

Time (mins)
B13: BIOMODEL; PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/L) vs. Time (mins)
B14: BIOMODEL; PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/l)

Time (mins)

0 10 20 30 40 50

0 5 10 15 20 25 30 35 40
B15: BIOMODEL: PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/l) vs. Time (mins)
B16: BIOMODEL; PREDICTED CONC. PROFILES OF BFB

- Nitrate
- Nitrite x10
- Methanol

Concentration (mg/l) vs Time (mins)