Denitrification of groundwater: pilot-plant testing of cotton-packed bioreactor and post-microfiltration

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Abstract: The use of raw cotton as carbon source in the denitrification of drinking water was tested in a field pilot-plant. The reactor treated water from a well in which the concentration of nitrate varied from 22 mg N l–1 in summer to a minimum of 9 mg l–1 in winter. The experimental reactor had a capacity of approximately 9 m3 and could be packed with up to 1500 kg of unprocessed cotton. The highest rate of denitrification observed was 0.36 kg N m–3 d–1, at a feed rate of 6 m3 h–1. However, this performance could be sustained only temporarily as the relatively high water pressure caused serious compression of the bed. The long-term (six months) performance of the system was studied at feed rates of 0.8 and 1.5 m3 h–1. The process was stable and 80–100% of the influent nitrogen was removed. The increase in DOC at the outlet was usually less than 7 mg l–1, and the number of bacteria was in the order of 105–106 CFU ml–1. Crossflow microfiltration was an effective post-treatment for the removal of bacteria and elimination of turbidity.

Keywords: Cellulose; cotton; denitrification; field pilot; microfiltration; water treatment

Introduction

Nitrate pollution of drinking water sources is a universal environmental problem, and a number of methods (biological, chemical, physical and physico-chemical) have been developed to lower the concentration of nitrate to levels acceptable for human consumption. Among them, microbial removal (denitrification) is considered to be the most economical and environmentally sound, as well as being feasible on a large scale. Biological denitrification is a process of reduction of nitrate to a gaseous product, preferably nitrogen gas, through a sequence of enzymatic reactions. Denitrifying bacteria have the capacity, under anaerobic conditions, to use nitrate in place of oxygen as a terminal electron acceptor in their respiratory processes. Biological treatments take advantage of this anaerobic respiratory process and aim to maximize the rates of nitrate consumption by assuring a steady supply of carbon and energy sources.

Refined simple organic compounds are commonly used as sources of energy for water denitrification, but cellulose-rich sources may offer an inexpensive alternative. Boussaid et al. (1988) and Avnimelech et al. (1993) have demonstrated the capacity of wheat straw to support denitrification of groundwater and wastewater. Cellulose is the most abundant renewable resource in the world and constitutes a high proportion of agricultural and domestic wastes. It is a linear glucose polymer with hydrogen bonding between hydroxyl groups of neighboring parallel chains organized in fibers imbedded in lignin and hemicellulose, an association which confers resistance to enzymatic degradation. In this respect, cotton is an exception in that it is the purest form of naturally occurring cellulose with only a small percentage of impurities, mostly in the form of wax (0.3–1%), pectin in the outer layers (0.5–1.2%), and protein residues. Cellulolytic microorganisms are widely distributed in nature. Initial cellulose degradation requires direct physical contact between the enzyme molecules and the surface of cellulose, and complete degradation depends on the concerted action of several enzymes (cellulases) which act in synergism.
Laboratory studies were recently carried out in order to assess the efficiency of cotton (Volokita et al., 1996a), newspaper (Volokita et al., 1996b), and straw (Soares and Abeliovich 1998) as substrates for denitrification of drinking water. Cotton was by far the most efficient substrate and was selected for further field study. Summarized here are the results obtained in a field pilot in which short fiber (low commercial value) unprocessed cotton served as the sole chemical and physical substrate for microorganisms. Post-treatment of the denitrified water was by crossflow membrane microfiltration.

Materials and method

Denitrification set up

Figure 1 describes the structure of the bioreactor, a steel cylinder, 210 cm diameter and 355 cm height, with domed cap and four sampling taps spaced along its height. This external cylinder enclosed a smaller one, 188 cm diameter and 320 cm high, perforated from the bottom up to 35 cm from the top; a small square of metal soldered at an upwards-facing angle protected each perforation from being clogged due to compression of cotton against the side walls. A pipe with a blind upper end and with side perforations (up to 35 cm from the top) was placed at the center of the internal cylinder which was packed with approximately 1.2 ton of unprocessed cotton (Gossypium hirutum). Water was pumped in through an inlet at the base and was distributed along the cotton bed through the perforated pipe. Denitrified water flowed to the space between the two cylinders and out through an outlet at the top of the external cylinder; a venting pipe placed higher on the upper dome allowed the escape of gases and prevented pressure buildup. An external pipe and an additional pump allowed recirculation between the top and the bottom of the reactor.

The bacterial inoculum was prepared as follows: approximately 100 g of wet cotton from an active laboratory column (Volokita et al., 1996a) were placed in a 50 l bucket which was then filled with cotton and tap water amended with 70 mg l⁻¹ nitrate-N, 9 mg l⁻¹ phosphate and 3 ml l⁻¹ of a microelements solution. After three days of incubation, the inoculum was added to the reactor which was then slowly filled with water and left in a recirculation mode for 3–5 days. The following water quality parameters were routinely monitored both in the influent and effluent: nitrate, nitrite, ammonia, pH, DOC, bacterial numbers (CFU) and turbidity.

Post-treatment – microfiltration set up

Post-treatment was by direct flow filtration with a MEMCOR (Memtec Limited, South Windsor, NSW, Australia) Type K00320 microcompact filtration unit with four filtration modules in parallel. Each module consisted of an encapsulated bundle of hollow fiber microporous polypropylene membranes (0.2 µm pore size and approximately 0.5 mm diam) with the nominal filtration area of 1 m² and an average permeate flux of about 500 l h⁻¹.
Periodic backwashing was carried out to remove colloid matter from the membrane surface and maintain high performance. This was achieved by reversing the direction of the raw water flow (via the feed tap) and by compressed air. Backwashing was usually done whenever an increase was measured in the resistance to flow in terms of transmembrane pressure (TMP), which represents the difference of pressure between the two sides of the membrane.

A microfiltration system typically operates at very low pressures of approximately 3–15 psi (10–50 kPa). During normal operation, the feed passes from the outside of the membrane (from the module shell) into the center (lumen) and exits as filtrate. Suspended solids and microorganisms are retained on the outside surface of the hollow fiber. Typical system feed pressure is 25 to 35 psi (170 to 240 kPa). The normal operating differential pressure for the membrane is 5 to 30 psi (35 to 210 kPa), with an average initial differential pressure loss of 5 to 8 psi (35 to 55 kPa).

Analytical determinations and bacterial counts

Nitrate was determined by the colorimetric method of Cataldo et al. (1975), and nitrite and ammonia were assayed according to Standard Methods (1992). Organic carbon was determined by means of a high-temperature TOC analyzer (Dohrmann DC-190, Rosemount Analytical Inc., Santa Clara, CA, USA). Turbidity was measured with a Hach (Loveland, Colorado) model 2100 P turbidimeter expressed as nephelometric turbidity units (NTU). Colony forming units were determined in diluted water samples by standard plating techniques on R2A agar (Difco Laboratories).

Results and discussion

Denitrification

Design of the field reactor was based on previous laboratory studies and on observations from a preliminary field test. The main considerations were: to maximize the distribution of the “active” zone, to minimize the accumulation of nitrogen gas, and to avoid compression of the bed by high water pressures and/or build up of gas pressure. In the plug-flow laboratory reactors denitrification occurred mainly in the lower half (Volokita et al., 1996a), however, in the reactor presented in Figure 1 water entered at the center and denitrification was expected to be equally distributed throughout the bed. Recirculation through the external loop was an additional measure to assure mixed conditions in the reactor. Spreading of the denitrification reaction throughout the bed was expected to result in an even distribution of gas and prevent the formation of high-pressure pockets of entrapped gas (Soares et al., 1989; 1991).

The feed rate above which compression of the bed might become a problem was unknown and it was decided to start the experiment at a relatively high flow rate. Thus, after the inoculation period, the reactor was started at the feed rate of 6 m$^3$ h$^{-1}$. Water samples were drawn after four days and by then no nitrogen was detected in the effluent (results not shown), representing a denitrification rate of 3.25 kg of nitrate per day or 0.36 kg N m$^{-3}$ d$^{-1}$. However, this performance lasted a few days only: internal high pressure developed, the bed was strongly compressed, and water flowed through channels of lower resistance only. As a result, the rate of denitrification declined considerably.

A new run was then initiated with the objective of testing the long-term performance of the system at lower feed rates. The feed rate was first lowered to 0.8 m$^3$ h$^{-1}$ and later increased to 1.5 m$^3$ h$^{-1}$ (a recirculation rate of approximately 0.8 m$^3$ h$^{-1}$ was maintained throughout the experiment). During the first 7 weeks, the concentration of nitrate in the well was close to 20 mg N l$^{-1}$. Later, due to winter rainfalls the concentration of nitrate decreased to 9–12 mg N l$^{-1}$. By mid-spring, the level of nitrate increased again and stayed at 18 mg N l$^{-1}$ until the end of the run (Figure 2a). Nitrate was never detected in the well water.
Complete or almost complete removal of nitrogen was observed during the first nine weeks of the run with the flow rate of 0.8 m$^3$/h\(^{-1}\) (Figure 2a and b). Then the feed rate was raised to 1.5 m$^3$/h\(^{-1}\) and an increase of nitrate washout followed (Figure 2a and b). This was due to serious depletion of cotton, and upon addition to the reactor of 800 kg cotton (2 March, 1996a).

### Table 1: Cost of electron donor for denitrification

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Cost/kg ($)</th>
<th>kg electron donor needed to remove 1 kg NO$_3^-$N</th>
<th>Cost/kg NO$_3^-$N ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.70</td>
<td>1.9$^a$</td>
<td>1.33</td>
</tr>
<tr>
<td>Acetate</td>
<td>1.67</td>
<td>2.7$^a$</td>
<td>4.37</td>
</tr>
<tr>
<td>Cotton</td>
<td>0.53</td>
<td>2.8$^b$</td>
<td>1.48</td>
</tr>
</tbody>
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$^a$Gommers (1987); $^b$Volokita et al. (1996a)

**Figure 2**: Concentrations of nitrate and nitrite (a), nitrogen loss (b and c), and pH and increase of DOC (d) in the field reactor operated at the feed rates of 0.8 and 1.5 m$^3$/h\(^{-1}\). Arrow indicates an addition of cotton which had an immediate effect on the rate of denitrification.
the rate of nitrogen removal increased up to 85% (Figure 2b). In May,
depletion of substrate again caused a drop in the rate of denitrification, which was also
remedied by the addition of fresh cotton (Figure 2a and b).

Except when cotton was depleted, breakthrough of nitrate was low. The concentration of
nitrite-N at the outlet was normally below 0.6 mg l–1 and reached a maximum of about
1.5 mg l–1 during periods of instability (Figure 2a). The amount of nitrogen removed per
day varied between approximately 0.45 and 0.2 kg d–1, corresponding to rates of 0.055 and
0.020 kg N m3 d–1 (Figure 2c). These rates of denitrification do not reflect the biological
capacity of the reactor as hydraulic constraints caused by the compression of the bed were
the major limiting factors, and the relatively large volume of the reactor was therefore not
efficiently exploited.

The increase in DOC was usually below 7 mg l–1, and higher values were seldom
observed (Figure 2d). The laboratory studies showed that degradation of cellulose was the
rate-limiting step in cotton-dependent denitrification and that significant amounts of DOC
were eluted only when nitrate was limiting (Volkita et al., 1996a). The pH of the effluent
was approximately 0.5 units lower than that of the influent (Figure 2d).

After denitrification, the number of colony forming bacteria in the water usually
increased by two orders of magnitude, from approximately 103 to 105 cells ml–1. These
numbers are well within the range reported for other biological systems (Soares et al.,

It was not possible in the field to determine the ratio of cotton consumed to nitrogen
removed. This value was determined previously in a laboratory experiment in which a reac-
tor was operated under a non-limiting supply of nitrate and the overall loss of nitrogen was
compared to the decrease in dry weight of the substrate; a cotton:N ratio of 2.8 was obtained
(Volkita et al., 1996a).

Costwise, cotton is a very attractive substrate for water denitrification. The less expen-
sive simple carbon source applied in water denitrification is methanol, but due to its toxici-
ty, the use of methanol is restricted to the treatment of wastewater. The least expensive
simple carbon source available for the treatment of drinking water is acetate. The cost of
cotton is approximately one third of that of acetate (Table 1).

Microfiltration
Microfiltration was investigated as a possible post-treatment strategy for the removal of
bacteria and turbidity. First, microfiltration delivering filtrate at low-pressure (0 atm) was
studied. The filtration unit was operated with untreated well water for 3 h in order to obtain
stabilized operating conditions to be used as the base line.

The feed supply was then changed from well water to reactor effluent while all operating
parameters remained unchanged. Automatic backwash was carried out every 15 minutes.
At regular intervals, pressures of the feed inlet and outlet and of the filtrate were recorded,
and the crossflow and the flux of the filtrate were measured.

<table>
<thead>
<tr>
<th>Table 2 Summary of microfiltration experiments</th>
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<tbody>
<tr>
<td>Run 1</td>
</tr>
<tr>
<td>Filtrate pressure (atm)</td>
</tr>
<tr>
<td>Crossflow (m3 h–1)</td>
</tr>
<tr>
<td>TMP (atm)</td>
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<tr>
<td>Steady flux (l m–2 h–1)</td>
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The run lasted 2 hours, during which the crossflow and the TMP were constant at 4.6 m$^3$h$^{-1}$ and 0.39 atm, respectively (Figure 3, run 1). Next, microfiltration delivering filtrate at high-pressure (0.3 atm) was tested in order to meet operational constraints related to the water supply system (Figure 3, run 2). The two runs can be summarized as shown in Table 2. The steady flux for the two runs was approximately the same. Nevertheless, as the filtrate pressure was higher during the second run, a loss of energy occurred which led to a crossflow drop. This crossflow drop increased membrane fouling leading to increase of the TMP which is detrimental to membrane operation. The conclusions taken from the microfiltration experiments were: (a) that the first set of operating conditions (0.39 atm TPM and 4.6 m$^3$h$^{-1}$ crossflow) was the best as the steady flux was nearly the same in both cases; and (b) that the membranes were not suited to support continuously 1 atm of TMP. A solution to having the higher pressure at the filtrate (0.3 atm) without membrane fouling would be to use a more powerful pump that would allow for a higher crossflow (around 4 or 6 m$^3$h$^{-1}$). In that case the TMP would be stable.

Microfiltration improved the quality of the denitrified water. The turbidity of the filtrate varied between 0.07 and 0.2 NTU while the turbidity of the reactor effluent ranged between 2 and 3 NTU; the turbidity of the well water was 0.69 NTU (Figure 4b). Microfiltration also decreased the number of bacteria (Figure 4a). It is reasonable to assume that hardly any bacteria passed through the 0.2 $\mu$m pores of the membranes, and that the bacteria found in the permeate were acquired through contact with pipes and the surrounding environment. The operating cost of microfiltration would be very low (a few cents per m$^3$) due to the low pressure applied and the low presence of suspended solids in the denitrified water.
Conclusions
The above work was the first step in the upscaling of a denitrification process developed in small (2.5–3.5 l) laboratory reactors (Volkita et al., 1996a). Although the design and operation of the field set-up remains to be optimized, the following conclusions can be drawn:
• Cotton is an inexpensive and efficient substrate for denitrification of nitrate-contaminated water.
• The cotton-dependent denitrification process is simple and feasible on a large scale.
• If the feed rate is kept within certain limits (which will vary with the size and configuration of the reactor), the performance of the system is reliable over long periods of time.
• Reactors smaller than the one in this trial should be used. When necessary, a system of parallel reactors could be employed.
• Microfiltration is a fast and effective method for the removal of bacteria and turbidity from the denitrified water.

Acknowledgements
This research was supported by Grant No. WT 9638 GR 1476 from the Ministry of Science and The Arts (MOSA), Israel, and the Bundesministerium fuer Bildung Wissenschaft, Forschung und Technologie (BMBF), Germany.

References