Biological denitrification of drinking water using various natural organic solid substrates

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Abstract Denitrification of drinking water was studied using various natural organic solid substrates (NOSS) such as poplar, hornbeam, pine shavings and wheat straw as a carbon source in a batch unit. The highest nitrate removal efficiency was observed with the wheat straw, so it was chosen as the carbon source for biodenitrification in an upflow laboratory reactor. In order to remove solid particles from the effluent water, a sand filter unit was placed after the denitrification reactor. The soluble DOC contents in the reactor affected the efficiency of nitrate elimination and nitrate concentration of the effluent water remained below acceptable values (50 mg/l NO₃⁻). In order to remove colour, DOC and nitrate from the water, powdered activated carbon adsorption studies were performed in the batch unit.

Keywords Biological denitrification; drinking waters; natural organic solid substrates

Introduction

A considerable increase in nitrate concentrations in drinking water has been observed in many countries, including Turkey (Aslan et al., 2001). Nitrate pollution of drinking water is caused mostly by excessive application of nitrogen-based fertilizers and irrigation with ammonia-rich effluents discharged by wastewater treatment plants. Increase in nitrate concentration in drinking water supplies causes methaemoglobinaemia in infants and cancer of the alimentary canal (Wasik et al., 2001) and there is no other group of carcinogens that can produce such a wide variety of tumors (Mirvish, 1991). In view of these health effects, the European Union, environmental agencies like the United States Environmental Protection Agencies (EPA) and the World Health Organisation (WHO) have set the maximum level of nitrate in drinking water at 50 mg/l.

It is necessary to reduce nitrate from drinking water supplies for human consumption when the nitrate concentration exceeds the drinking water standards. There are several methods for nitrate removal from drinking water supplies such as ion exchange, biodenitrification, reverse osmosis, electrodialysis and distillation. Among the various methods for nitrate removal from drinking water supplies, biological processes have been shown to be more efficient and convenient (Green et al., 1994; Volokita et al., 1996a; Bandpi and Elliot, 1996).

The majority of microbial denitrification treatment relies on heterotrophic bacteria, which require an organic carbon source; but drinking water has low carbon content. Therefore an external carbon source has to be supplied for microbial growth. The external carbon sources for nitrate removal from drinking water such as methanol (Hoek and Klapwizk, 1987; Gomez et al., 2000; Lee et al., 2001; Wasik et al., 2001), ethanol (Green et al., 1994; Dahab and Sirigina, 1994; Delanghe et al., 1994; Bandpi and Elliot, 1996; Gomez, et al., 2000; Fonseca et al., 2000; Aslan, 2002) and acetic acid (Dahab and Kalagari, 1996; Bandpi et al., 1999) have been widely used in laboratory studies. The possibility of using alternative substances such as volatile fatty acids (Yatong, 1996), shredded
newspaper (Volokita et al., 1996a), wheat straw (Soares and Abeliovich, 1998), unprocessed short fibre cotton (Volokita et al., 1996b), atrazine (Stucki et al., 1995), natural gas methane (Rajapakse and Scutt, 1999), elemental sulfur (Eisentraeger et al., 2001; Soares, 2002), sugar or glucose syrup and sugar cane (Nurizzo and Mezzanatte, 1992; INCO-DC, 2000) have also been studied for the biological denitrification processes.

In this study, poplar, hornbeam shaving waste materials and wheat straw were used in order to determine the suitable natural organic solid carbon source (NOSS) for the biological denitrification process. The performances of the various NOSS were investigated for about three months by batch experiments. The highest nitrate removal efficiency was observed with the wheat straw. Therefore wheat straw was chosen as the carbon source for biodenitrification in the following experiments.

Materials and methods

Biodenitrification batch experimental study

The microorganisms used in the experiments were developed in a laboratory scale biological denitrification unit. The culture was acclimated to the media for 2 weeks before using in denitrification experiments. Enrichment cultures were prepared in 250 ml Erlenmeyer flasks with medium solution containing 100 mg/l NO$_3^-$ (as NaNO$_3$) and 3 mg/l phosphate (as Na$_2$HPO$_4$ x $2H_2$O).

500 mg NOSS was placed in 250 ml Erlenmeyer flasks containing medium solutions. All flasks were sterilised in an autoclave for half an hour. Microorganisms were added to the flasks and cultures were placed on a labquake incubator at 27°C and flasks were shaken manually two times a day. Samples were drawn periodically and analysed for nitrate, nitrite and dissolved organic carbon (DOC). After 19 days, when nitrate elimination were not observed any more, 500 mg NOSS were added in order to supply a carbon source for microorganisms. Nitrate and phosphate were supplemented by addition of an appropriate volume from concentrated stock solution containing 1,000 mg/l nitrate and 30 mg/l phosphate.

Biodenitrification in continuous experimental study

Experimental apparatus. The experimental set-up consisted of a cylindrical glass biodenitrification unit with 13 cm inner diameter and 14.5 cm height. The sand filter column was 8 cm in diameter and 30 cm in height. The sand filter column was filled with filter sand of an effective diameter of 0.5 mm and uniformity coefficient of 1.23. The stainless steel sieves were placed at the inlet and the exit of the reactor to prevent washout of wheat straw fragments.

The continuous biodenitrification reactor packed with 150 g wheat straw was inoculated with microorganisms taken from the wheat straw batch unit. The column was fed with pure water containing 100 mg/l nitrate and 3 mg/l phosphate. The flow rate was regulated using a peristaltic pump and the column was operated in upflow mode. The inoculation lasted 3 days with daily replenishment of nitrate and phosphate. The column was allowed to stand for 3 days before the flow was initiated.

Samples were collected daily from the inlet and outlet of the biodenitrification reactor and sand filtration column and were routinely analysed for nitrate, nitrite, DOC, colour, dissolved oxygen, and pH. Experimental study was carried out at room temperature (31 ± 1°C in the summer).

Adsorption experiment with PAC

To remove colour and excess DOC, a powdered activated carbon (PAC) adsorption study was performed. In this study, 15 g wheat straw was soaked in 2 litre pure water overnight at
room temperature (31°C). Constant volumes of liquids containing 190 mg DOC/l and 270 Pt-Co colour were then supplemented with a series of known weights of PAC in glass Erlenmeyer flasks and the slurries were agitated at constant stirring velocity (125 rpm) on a shaker. Prior to use, the PAC was oven-dried at 105°C for 2 h and then cooled in a desiccator.

**Analytical methods**
Nitrate and nitrite-nitrogen were determined using analytical kits for nitrate (14773) and nitrite (14776) and a photometer Merck SQ 300 for the batch test. Nitrate was measured according to the brucine method (APHA, 1971) for continuous study. Colour was measured using an alpha platinum-cobalt standard (Heck Chemical Company). Dissolved oxygen (DO) measurements were carried out using an oxygen meter (WTW). DOC was determined using a TOC analyser (Dohrmann DC-190).

**Results and discussion**

**Biodenitrification batch study**
The overall amount of nitrate removed in the 250 ml batch study were about 30 mg, 40 mg, 60 mg and 185 mg for poplar, hornbeam, pine shaving and wheat straw, respectively (Figure 1–4). Nitrogen elimination was relatively higher for wheat straw and pine shaving. Because of the low carbon content of the poplar and hornbeam shaving (about 60 mg/l), significant nitrate elimination was not observed.

Using hornbeam, 23 mg NO₃⁻ elimination was obtained when the remaining DOC concentration was about 27 mg/l. After the addition of 500 mg hornbeam, cumulative nitrate elimination increased to 40.5 mg and the remaining DOC concentration was 36 mg/l, which could not be used by microorganisms. Nitrite was not observed in the batch unit except for the first sample (Figure 1).

![Figure 1](http://iwaponline.com/wst/article-pdf/48/11-12/489/422110/489.pdf)

**Figure 1** Nitrate elimination, nitrite and DOC contents in batch unit containing hornbeam shaving

![Figure 2](http://iwaponline.com/wst/article-pdf/48/11-12/489/422110/489.pdf)

**Figure 2** Nitrate elimination, nitrite and DOC contents in batch unit containing poplar shaving
On 8th day, the cumulative nitrate removal was 9 mg in the poplar batch unit (Figure 2). After adding extra poplar, DOC content was increased from 33.5 to 45.5 mg/l on the 20th day. Nitrate elimination was increased to 30 mg and DOC content decreased to 15.7 mg/l at the end of the study. On the first days of operation, nitrite concentration did not exceed 1.5 mg/l and after 8 days, nitrite was not observed.

The DOC level of wheat straw and pine shaving were about the same at the beginning of the batch experiment (Figure 3 and 4). However, low rate of nitrate removal and high nitrite accumulation was observed with pine shaving. Nitrite concentration did not drop below 13 mg/l and about 66 mg DOC/l remained in the batch unit. This might be due to the hard breakdown of the carbon chain by microorganisms.

The highest nitrate removal was observed with the wheat straw. The soluble fraction of carbon present in the wheat straw allowed a rapid microbial growth and therefore high nitrate removal was observed in the batch unit. During the six-day period, microorganisms consumed about 57% of the soluble fraction of DOC and 63 mg nitrate was eliminated. Nitrite accumulation was observed in the first day of operation, but later it decreased gradually from 14 mg/l to below detection limit. After adding extra wheat straw, nitrate elimination was enhanced to 129 mg on the fourth day. Nitrite was not observed except after 73 days for operation. Due to the low DOC content, nitrate elimination was not determined after 40 days.

During autoclaving, wheat straw releases carbon fractions, some of which are biodegradable and some are not. In the first step of the study when DOC concentration decreased to 56.5 mg/l, nitrate elimination was not observed any more. However after adding DOC in the second step, nitrate elimination has started and continued down to the level of 22.5 mg DOC/l. It can be assumed that the consumable fraction of the DOC enhanced the microbial activity. When wheat straw was used as carbon source for biological denitrification, all the water soluble component and a significant portion of the cellulose and hemicelluloses had been lost by the end of the experiment, while lignin and mineral components remained unchanged (Soares and Abeliovich, 1998).

![Figure 3](https://iwaponline.com/wst/article-pdf/48/11-12/489/422110/489.pdf)  
**Figure 3** Nitrate elimination, nitrite and DOC contents in batch unit containing pine shaving

![Figure 4](https://iwaponline.com/wst/article-pdf/48/11-12/489/422110/489.pdf)  
**Figure 4** Nitrate elimination, nitrite and DOC contents in batch unit containing wheat straw
As a result of the batch study, wheat straw was selected as the carbon source and support medium for the biological denitrification reactor for the continuous study.

**Biodenitrification continuous reactor experiments**

A column packed with 150 g of wheat straw was operated since nitrate removal efficiency was lower than 50% when DOC concentration was too low. High nitrogen removal was observed during the first days of operation, but high DOC containing colourful water was washed out from the biological reactor (Figure 5). The easily soluble fraction of carbon causes rapid microbial growth therefore high nitrate removal efficiencies were observed. A colour of about 500 Pt-Co unit and odour were observed during the inoculation period because of the high temperature (about 31°C) in summer. Dissolved oxygen concentration did not exceed 0.3 mg/l in the biological reactor.

During the continuation of the study, the water velocity ranged between 0.02–0.1 m/h and effluent water pH between 6.7–7.1 for reactor and 6.5–6.7 for sand filter. As more water passes through the reactor, a decrease in colour and DOC content of the reactor effluent was observed. In fact, the concentration of DOC was 400 mg/l, colour was about 500 Pt-Co during the inoculation period and decreased gradually to about 3 mg DOC/l and 10 Pt-Co at the end of the study.

Using wheat straw, significant nitrate elimination was determined up to the point where effluent water contains about 7 mg DOC/l and that NO₃⁻ concentration was 42.1 mg/l, which is well below the acceptable level for drinking water. After 100 litre of water was passed through the reactor, due to the consumption of the soluble fraction of wheat straw, nitrate elimination slowed down and drinking water standard was exceeded. During the operation period, 23.5% of the initial weight of wheat straw was lost and 18.8 g of it was exhausted per g of nitrogen removed. The wheat straw consumption reported in this study is higher than the values given in the literature for denitrification with wheat straw (Soares and Abeliovich, 1998).

The effluent from the biological denitrification reactor was sent to the sand filtration unit where suspended solids in the water were removed. Using the sand filter system, between 16–22% nitrate removal efficiencies were obtained when DOC content was sufficient for microbial denitrification in the filter (Figure 6). If the effluent water contains sufficient carbon and nitrate, denitrification occurs at the sand filter system. Under these conditions the sand filtration unit behaves like a biodenitrification reactor.

Post treatment is needed to remove colour in the effluent water because it includes high DOC and colour, most pronounced, especially in the first days of operation. To remove excess DOC and colour from the effluent, adsorption study was performed using PAC.
result of the batch studies, complete colour removal was achieved with 1 g PAC for 100 ml of water containing colour (270 Pt-Co). Fifty two per cent DOC removal (from 190 mg/l DOC to 91 mg/l) was obtained with 1.5 g PAC. For further study, which was carried out at different agitation times, 1.5 g PAC was used. In this series 100 mg NO₃⁻/l was supplied to the water containing 190 mg DOC/l. In order to reduce DOC content in the effluent, PAC adsorption is required. Even though high concentration of DOC was washed from the biological denitrification and sand filter system; a considerable amount of DOC and nitrate might be removed using PAC. As can be seen from Figure 7, 63% nitrate and 98% DOC removal efficiencies were observed in 24 hours.

Conclusions
Since nitrate pollution of drinking water is encountered mainly in rural areas, this study has been performed considering the water treatment plants of small communities (i.e. villages). The following conclusions may be derived from the studies.
1. Wheat straw is a more suitable carbon source than other NOSS tested. Although pine shavings and wheat straw have about the same carbon content at the beginning of the batch studies, the highest nitrate elimination was obtained with wheat straw. This might be due to the easy breakdown of the carbon chain of the wheat straw by denitrifying microorganisms.
2. In order to improve effluent water quality, PAC adsorption is recommended to remove the remaining DOC, colour and nitrate.
3. A sand filtration unit improves the water quality with respect to suspended solids. In case high concentration of carbon and nitrate is fed, sand filtration also acts as a bi-denitrification unit and considerable nitrate removal is observed.
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References


