review

# Biological water denitrification—A review

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*Different methods of biological denitrification of drinking water as well as the microbiology and stoichiometry of the process are reviewed. Autotrophic bacteria oxidizing anorganic compounds or heterotrophic species oxidizing organic materials can perform denitrification. Generally, autotrophic bacteria grow slowly and denitrification rates are lower, whereas contamination of denitrified water with organic materials requires extensive posttreatment in the heterotrophic processes. Most full-scale applications make use of heterotrophic processes due to their efficiency, high specific denitrification rate, and operational simplicity. Both processes have been tried also* in situ *using soil infiltration, but underground phenomena remain difficult to control.* In situ *denitrification is applicable only under certain geological conditions.*

**Keywords:** Biological denitrification; drinking water; microbiology; nitrate removal; stoichiometry; bioreactor; unit process

## Introduction

Nitrate contamination of groundwater resources is becoming a problem in Europe as well as in the United States and Canada. In many areas the nitrate concentration in groundwater has reached serious levels exceeding the nominal limits of 10.0 mg $l^{-1}$ as $NO_3^-$-N (nitrate nitrogen) set by the U.S. Environmental Protection Agency[1,2] or 50 mg $l^{-1}$ as $NO_3^-$ (nitrate) set by the World Health Organization, the European Economic Community, and some former East European countries, e.g. Czechoslovakia.[3–5] Concern over increase in nitrate concentrations is very legitimate due to potential ill effects on health. The toxicity of nitrates for humans is not clearly established. However, their consumption can cause infant methemoglobinemia (blue baby syndrome).[6] Reduction of nitrates into nitrites in saliva may contribute to the formation of nitrosoamines, which are known carcinogens.[7,8]

Three methods show some potential for full-scale application: ion exchange, reverse osmosis, and biological denitrification.[2,9]

Ion exchange is limited by two problems. The first is that a resin of high selectivity for nitrates over ions that are commonly present in groundwater does not exist. The second problem involves providing an adequate resin regenerant such that regenerant disposal does not become a problem itself.[10,11]

The problem of reverse osmosis is that the membranes used generally do not exhibit high selectivity for nitrates. The degree of salt rejection is directly related to the valency of the ions. That is why the reverse osmosis process results in better removal of multivalent ions. Reverse osmosis results in the removal of many ionic species and in a significant reduction in the mineral content of the water.[2,12]

The most promising and versatile approach being studied is biological denitrification. This process has been used for years in wastewater treatment.[13–19] Biological denitrification is highly selective for nitrate removal. The efficiency of the process is very high and can reach nearly 100%, which is not matched by any other methods available for nitrate reduction.[2] The potential bacterial contamination of treated water is the main disadvantage. This risk is very legitimate and subsequent treatment and disinfection are required to meet current drinking water standards.[20–22]

## Microbiology

Many bacteria are capable of growing anaerobically by reducing ionic nitrogenous oxides to gaseous products.[23–26] This respiratory process, in which nitrates or nitrites serve as terminal electron acceptors instead of oxygen, results in generation of ATP[27,28] and is termed denitrification or dissimilatory nitrate reduction.[29–33] When electrons are transferred from the donor to the

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**Table 1** List of bacterial genera that are known to contain denitrifying species

| Bacterial genus | Ref. |
|---|---|
| *Achromobacter* | 14,40 |
| *Acinetobacter* | 41–43 |
| *Aeromonas* | 44 |
| *Alcaligenes* | 42,45,46 |
| *Aquaspirillum* | 47 |
| *Azospirillum* | 48–52 |
| *Bacillus* | 44,53 |
| *Beggiatoa* | 54 |
| *Chromobacterium* | 55,56 |
| *Clostridium* | 57 |
| *Desulfovibrio* | 58 |
| *Erythrobacter* | 59 |
| *Gallionella* | 60 |
| *Halobacterium* | 26 |
| *Halomonas* | 61 |
| *Hyphomicrobium* | 34 |
| *Janthinobacterium* | 62 |
| *Neisseria* | 56 |
| *Paracoccus* (formerly *Micrococcus*) | 63–65 |
| *Propionibacterium* | 66,67 |
| *Pseudomonas* | 68–72 |
| *Rhizobium* | 73–75 |
| *Rhodobacter* (formerly *Rhodopseudomonas*) | 76–79 |
| *Thiobacillus* | 80–84 |
| *Thiosphaera* | 39,85 |
| *Vibrio* | 86 |
| *Xanthomonas* | 87,88 |

acceptor, the organism gains energy which can be applied for the synthesis of a new cell mass and the maintenance of the existing cell mass.

The most detailed investigations into denitrification have involved a limited group of specialized bacteria. This has resulted in a view that denitrification can only occur under anaerobic conditions. However, it has been shown that denitrification can occur in the presence of oxygen in certain species too.[34–39]

Most of the organisms known to denitrify belong to bacterial genera listed in *Table 1*.

The enzymes associated with denitrification are synthetized when conditions become advantageous for denitrification.[89] Synthesis of denitrifying enzymes is typically a highly regulated process. It is generally held that the denitrifying enzymes are inducible. Their synthesis occurs under anaerobic conditions,[90] although denitrification can occur in the presence of oxygen.[35,36,91–95] In some cases enzyme induction may even require low concentrations of oxygen.[38,96] Significant levels of enzyme may be present as a consequence of anaerobiosis even in the absence of nitrate or other nitrogenous oxides.[27,97]

Reduction of nitrate to nitrogen gas proceeds in four steps according to the following scheme:[29,98]

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (1)$$

Each step is catalysed by an enzyme system. Dissimilatory reduction of nitrate to nitrite is important to a number of bacteria, since the process involves energy conservation through generation of a proton motive force[99,100] or by increased substrate-level phosphorylation reaction.[57] This step is catalyzed by membrane-bound nitrate reductases. The dissimilatory reduction of nitrite is carried out by two distinct nitrite reductases:[101–103] one contains a Cu center and the other contains two hemes. Both seem to carry out the same physiological reaction.[104] Nitric oxide is typically produced from nitrite, but under some conditions nitrous oxide is also produced.[90,105] Nitrite reductases are membrane-bound as well as cytoplasmatic enzymes.[108] The reduction of nitric oxide is the least well-characterized of the enzymatic steps associated with denitrification. But there are observations that support the notion that nitric oxide reductase is present in bacterial membranes.[107–109] The fractions that exhibited nitric oxide reduction to nitrous oxide were isolated from membranes of *Pseudomonas halodenitrificans*.[107] Shapleigh *et al.*[108] examined the production of nitrous oxide from nitrite by membranes prepared from various bacterial strains. They also concluded that nitrite reduction to nitrous oxide proceeds via nitric oxide and is catalysed by two discrete enzymes, a nitrite reductase and a nitric oxide reductase. The last denitrification step, the reduction of nitrous oxide to dinitrogen, is catalysed by nitrous oxide reductase. This step is coupled to ATP formation.[110–112] Several lines of evidence suggest that the enzyme is a copper protein[113–115] and is a cytoplasmatic one.[116]

While considerable progress has been made in characterizing denitrification enzymes and much is known about the mechanism of denitrification, several areas still remain to be clarified. The mechanism by which the N–N bond of nitrous oxide is formed has still to be resolved, even though the reactions have been studied intensively.[117,118]

From detailed studies of the enzyme systems of some bacterial species it can be concluded that factors affecting induction and repression of these enzymes are not universal because denitrifying bacteria as a group are genetically diverse and metabolically versatile. The influence of oxygen concentration,[119–125] pH,[66,126–128] temperature,[120,129] electron donor, and nitrate and intermediate concentrations[71,94,95,130–137] on denitrification performance of some bacterial species has been investigated in detail. On the contrary, very little is known about regulatory interdependence of the reductase involved.

The enzymology and mechanism of biological denitrification have been dealt with in more detail in other reviews.[90,138–140]

## Stoichiometry

Since denitrification is a respiratory process, an oxidizable substrate or electron donor is needed as an energy source. Denitrifying bacteria are mostly heterotrophic ones and utilize complex organic substances as oxidizable substrates: some are capable of utilizing 1-carbon compounds. However, some denitrifying bacteria are autotrophic and utilize hydrogen and carbon dioxide or reduced sulfur compounds. One group is photosynthetic.

During denitrification, nitrate (an electron acceptor) is reduced to gaseous nitrogen in accordance with the following general equation:[141]

$$0.2\ NO_3^- + 1.2\ H^+ + e^- \rightarrow 0.1\ N_2 + 0.6\ H_2O \quad (2)$$

Reduced organic carbon or reduced sulfur compounds are oxidized, which can be generally represented as:[141]

$$0.25\ CH_2O + 0.25\ H_2O \rightarrow 0.25\ CO_2 + H^+ + e^- \quad (3)$$

$$0.5\ FeS_2 + 4\ H_2O \rightarrow SO_4^{2-} + 0.5\ Fe^{2+} + 8\ H^+ + e^- \quad (4)$$

These equations do not include consumption of substrates for cell mass synthesis and maintenance.

### *Heterotrophic denitrification*

Heterotrophic denitrifying bacteria require an organic carbon source for respiration and growth. A wide variety of organic compounds has been used, such as methanol, ethanol, glucose, acetate, aspartate, or formic acid[20,32,130,131,142–146] as well as different industrial wastes including molasses, whey, distillery stillage, and sulfite waste liquor.[147–149] However, most of the published research regarding drinking water denitrification involves the use of methanol, ethanol, and acetic acid. If methanol is used as a carbon source, the stoichiometric relationships describing bacterial energy reactions in two steps are written as follows:[20,150]

$$6\ NO_3^- + 2\ CH_3OH \rightarrow 6\ NO_2^- + 2\ CO_2 + 4\ H_2O \quad (5)$$

$$6\ NO_2^- + 3\ CH_3OH \rightarrow 3\ N_2 + 3\ CO_2 + 3\ H_2O + OH^- \quad (6)$$

Overall energy reaction:

$$6\ NO_3^- + 5\ CH_3OH \rightarrow 3\ N_2 + 5\ CO_2 + 7\ H_2O + OH^- \quad (7)$$

Whereas these equations reveal the stoichiometric quantity of methanol required for nitrate dissimilation, additional methanol is required for deoxygenation and cell synthesis according to the following equations:[151]

$$O_2 + 0.93\ CH_3OH + 0.056\ NO_3^- + 0.056\ H^+ \rightarrow 0.056\ C_5H_7NO_2 + 0.65\ CO_2 + 1.69\ H_2O \quad (8)$$

$$14\ CH_3OH + 3\ NO_3^- + 4\ H_2CO_3 \rightarrow 3\ C_5H_7NO_2 + 20\ H_2O + 3\ HCO_3^- \quad (9)$$

The cell formula $C_5H_7NO_2$ suggested by Hoover and Porgess[152] was used.

On the basis of laboratory data, the following overall nitrate-removal reaction was developed:[153]

$$NO_3^- + 1.08\ CH_3OH + H^+ \rightarrow 0.065\ C_5H_7NO_2 + 0.467\ N_2 + 0.76\ CO_2 + 2.44\ H_2O \quad (10)$$

In practice, 25 to 30% of the methanol required is used for bacterial cell synthesis. When dissolved oxygen is present, the methanol requirement is correspondingly higher.[20,153] Therefore a common working value is 3.0 kg of methanol per kg $NO_3^-$-N removed rather than the stoichiometric amount, 2.47 kg of methanol.

Many other carbonaceous compounds have been studied as substrates for denitrification. The equations revealing the stoichiometric quantity of various organic carbon substrates required for nitrate dissimilation are listed in *Table 2*.

On the basis of experimental results, formulas have been developed that determine the requirement of various carbon sources for denitrification. Because the dependence of the amount of denitrified nitrogen on the concentration of the carbon source in the medium is linear, the formulas can be written as follows:

For methanol:[160]

$$c_{met} = 2.47\ NO_3^-\text{-N} + 1.53\ NO_2^-\text{-N} \quad (11)$$

For ethanol:[161]

$$c_{et} = 2.00\ NO_3^-\text{-N} + 1.28\ NO_2^-\text{-N} \quad (12)$$

**Table 2** Stoichiometric relationships of heterotrophic denitrification with various carbonaceous substrates

| Substrate | Stoichiometric equation | Ref. |
|---|---|---|
| Ethanol | $5\ C_2H_5OH + 12\ NO_3^- \rightarrow 10\ HCO_3^- + 2\ OH^- + 9\ H_2O + 6\ N_2$ | 150,154 |
| | $0.613\ C_2H_5OH + NO_3^- \rightarrow 0.102\ C_5H_7NO_2 + 0.714\ CO_2 + 0.286\ OH^- + 0.980\ H_2O + 0.449\ N_2$ | 154 |
| Acetic acid | $5\ CH_3COOH + 8\ NO_3^- \rightarrow 8\ HCO_3^- + 2\ CO_2 + 6\ H_2O + N_2$ | 146,154 |
| | $0.819\ CH_3COOH + NO_3^- \rightarrow 0.068\ C_5H_7NO_2 + HCO_3^- + 0.301\ CO_2 + 0.902\ H_2O + 0.466\ N_2$ | 154 |
| Propanol | $0.278\ C_3H_7OH + NO_3^- \rightarrow 0.5\ N_2 + 0.833\ CO_2 + 0.611\ H_2O + N_2$ | 150 |
| Cellulose | $5(C_6H_{10}O_5)_n + 24n\ NO_3^- \rightarrow 6n\ CO_2 + 13n\ H_2O + 12n\ N_2 + 24n\ HCO_3^-$ | 155 |
| Butanol | $0.208\ C_4H_9OH + NO_3^- \rightarrow 0.5\ N_2 + 0.833\ CO_2 + 0.542\ H_2O + OH^-$ | 150 |
| Pentanol | $0.167\ C_5H_{11}OH + NO_3^- \rightarrow 0.5\ N_2 + 0.833\ CO_2 + 0.5\ H_2O + OH^-$ | 150 |
| Glycol | $0.5\ (CH_2OH)_2 + NO_3^- \rightarrow 0.5\ N_2 + CO_2 + H_2O + OH^-$ | 151 |
| Aromatic hydrocarbons | $C_{61}H_{67} + 62.2\ H^+ + 62.2\ NO_3^- \rightarrow 31.1\ N_2 + 61\ CO_2 + 64.6\ H_2O$ | 156 |
| "Typical" organic matter | $C_5H_9NO + 3.36\ NO_3^- + 3.92\ H^+ \rightarrow 1.68\ N_2 + 0.36\ C_5H_7NO_2 + 3.2\ CO_2 + 3.92\ H_2O + 0.64\ NH_4^+$ | 157 |
| Methane | $5\ CH_4 + 8\ NO_3^- + 8\ H^+ \rightarrow 4\ N_2 + 5\ CO_2 + 14\ H_2O$ | 158 |
| Glucose | $C_6H_{12}O_6 + 2.8\ NO_3^- + 0.5\ NH_4^+ + 2.3\ H^+ \rightarrow 0.5\ C_5H_7NO_2 + 1.4\ N_2 + 3.5\ CO_2 + 6.4\ H_2O$ | 159 |

**Table 3** Stoichiometric relationships of autotrophic denitrification with various electron donors

| Electron donor | Stoichiometric equation | Ref. |
|---|---|---|
| Hydrogen | $2\ NO_3^- + 5\ H_2 \rightarrow N_2 + 4\ H_2O + 2\ OH^-$ | 166 |
| Ferrous iron | $NO_3^- + 5\ Fe^{2+} \rightarrow 0.5\ N_2 + 5\ FeOOH + 9\ H^+$ | 168 |
| Sulfide | $14\ NO_3^- + 5\ FeS_2 + 4\ H^+ \rightarrow 7\ N_2 + 10\ SO_4^{2-} + 5\ Fe^{2+} + 2\ H_2O$ | 81,141 |
| Thiosulfate | $8\ NO_3^- + 5\ S_2O_3^{2-} + H_2O \rightarrow 4\ N_2 + 10\ SO_4^{2-} + 2\ H^+$ | 163 |
| | $0.141\ NO_3^- + 0.125\ S_2O_3^{2-} + 0.0643\ CO_2 + 0.1\ H_2O \rightarrow 0.0129\ C_5H_7NO_2 + 0.064\ N_2 + 0.25\ SO_4^{2-} + 0.109\ H^+$ | 167 |
| Elemental sulfur | $10\ NO_3^- + 11\ S^0 + 4.1\ HCO_3^- + 0.5\ CO_2 + 1.71\ NH_4^+ + 2.54\ H_2O \rightarrow 0.92\ C_5H_7NO_2 + 11\ SO_4^{2-} + 5.4\ N_2 + 9.62\ H^+$ | 170 |

For acetic acid:[162]

$$c_{ac} = 3.30\ NO_3^-\text{-}N + 2.08\ NO_2^-\text{-}N \quad (13)$$

A comparison of the above formulas shows that the weight ratio of substrate to nitrogen is the most favorable in the case of ethanol. The C : N ratio for ethanol is 1.05 for nitrates and 0.67 for nitrites, whereas the respective values for methanol are 0.93 and 0.57 and for acetate 1.32 and 0.83. Thus, in terms of C : N ratio, ethanol is almost as effective in denitrification as methanol.[161] The additional losses of carbon source have to be included when dissolved oxygen is present.

### *Autotrophic denitrification*

Some bacteria from the genera *Paracoccus, Thiobacillus, Thiosphaera,* and others can accomplish denitrification autotrophically using hydrogen or various reduced sulfur compounds such as $S^0$, $S^{2-}$, $S_2O_3^{2-}$, $S_4O_2^{2-}$, or $SO_3^{2-}$ as energy sources.[83,163–167] Bacteria from the genera *Ferrobacillus, Gallionella, Leptothrix,* and *Sphaerotillus* can utilize ferrous iron as an energy source for autotrophic denitrification.[60,82,168–170] Under autotrophic growth conditions, carbon dioxide or bicarbonate is used as a carbon source for microbial cell synthesis.

*Paracoccus denitrificans* can denitrify using hydrogen when an organic carbon source is not present. *Thiobacillus denitrificans* and *Ferrobacillus ferrooxidans* use reduced sulfur compounds and ferrous iron, respectively, as energy sources. *Paracoccus denitrificans* and *T. denitrificans* can grow heterotrophically if an organic carbon source is present.[163–165] *F. ferrooxidans* is strictly autotrophic and utilizes the $CO_2$ as a source of carbon.

Stoichiometric equations of autotrophic denitrification with various energy sources are listed in *Table 3.*

It has been shown that the S : N ratio strongly influences autotrophic denitrification. When the S : N ratio is low, the accumulation of nitrite occurs because nitrate conversion to dinitrogen is limited. The minimum requirement of S : N was calculated to be 4.30 when thiosulfate was used as energy source. Under conditions of sufficient S : N ratio, no nitrite accumulation was observed.[167]

Theoretical stoichiometric coefficients for sulfur consumption per $NO_3^-$-N removed, calculated from stoichiometric equations for thiosulfate and elemental sulfur, were 3.86 and 2.55, respectively. Distinct differences in the coefficient values did arise from the species of reduced sulfur compounds employed. When the data obtained during continuous and batch denitrification experiments using elemental sulfur were used, the respective values of the stoichiometric coefficients increased to 3.3 and 3.0.[80] The slight increase could be ascribed to the partial aerobic oxidation of elemental sulfur.

The theoretical consumption of hydrogen is 0.35 mg $H_2$ per 1 mg $NO_3^-$-N reduced to dinitrogen. The experimental $H_2$ requirement was slightly higher than the stoichiometric values and ranged from 0.38 to 0.40 mg $H_2$ per 1 mg $NO_3^-$-N reduced.[167,171]

## *In situ* biological denitrification

Denitrification in natural systems without addition of an electron donor proceeds very slowly, and it is not significant for lowering of nitrate concentrations in aquifers.[171–176] That is why several investigators have evaluated the injection of various substrates and nutrients into aquifers to enhance *in situ* biological denitrification. Denitrification processes with organic carbon such as methanol, ethanol, or sucrose have been reported.[177–186] Denitrification efficiency has been reported in a wide range between 10% and nearly 100%.[177,179,182]

Rudovský *et al.*[179,186] and Rudovský and Janda[183,184] reported the performance of an *in situ* denitrification using ethanol as a substrate in Czechoslovakia. Ethanol was injected into the aquifer through eight injection wells located around the supply well at a distance of 10 to 15 m. The water was recycled between two neighboring injection wells by pumping to ensure good distribution of ethanol in the aquifer. The recycling of water in the amount of approximately 10 $m^3\ h^{-1}$ had a very good influence upon the homogeneous distribution of the substrate in the aquifer. Such an arrangement considerably improved the quality of denitrified water in comparison with an arrangement without recycling.

The full-scale *in situ* denitrification (capacity 36 $m^3\ h^{-1}$) has been operated for more than 3 years.[184] An

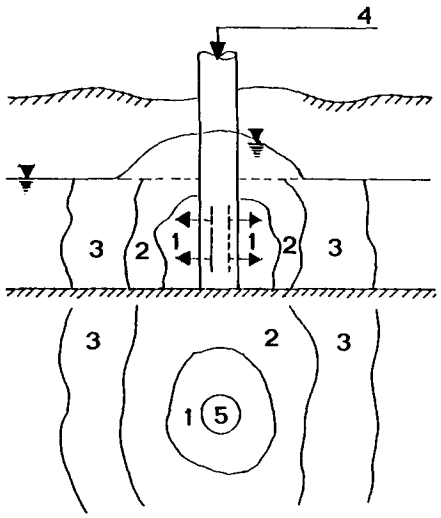


**Figure 1** Schematic description of a dual-purpose well for *in situ* denitrification. 1, Natural bioreactor; 2, sand filter; 3, denitrified water; 4, recharge flow amended with carbonaceous source and phosphate; 5, well

average nitrate removal of about 75% (130 mg $l^{-1}$ $NO_3^-$ in raw water; 30 mg $l^{-1}$ in denitrified water) was achieved. The content of bacteria in denitrified water is very close to the maximum permissible counts given by standards for drinking water quality, so that the subsequent treatment and disinfection of denitrified water is required. The subsequent treatment consisted of aeration, activated carbon filtration, and chlorination. No serious clogging problems were encountered during the study.

Mercado *et al.*[182] evaluated two alternative schemes for *in situ* biological denitrification in the Shivat Zion well, Israel. The organic substrate used was an aqueous solution of sucrose. Small amounts of phosphoric acid were added to supply the phosphorus required for the bacterial synthesis. The sucrose was dosed in such amounts that a slight excess to stoichiometric requirements was provided.

The first scheme tested was a dual-purpose well demonstrated in *Figure 1*. The principle of this scheme is an intermittent operation in the same well, consisting of recharge of nitrate-polluted water amended with substrate and consequent pumping of denitrified water. During pumping the nitrate concentration steadily rose in treated water. At the beginning of pumping the $NO_3^-$-N concentration was 3.5 mg $l^{-1}$; after withdrawal of approximately 1000 $m^3$ of water the $NO_3^-$-N content reached a background concentration of 13.5 mg $l^{-1}$. Nitrate removal efficiency of about 50% has been achieved. The microbiological quality of denitrified water was not good because of a high coliform bacteria count of $10^4$ to $10^5$ per 100 ml. Therefore subsequent treatment and disinfection were inevitable.

The Daisy system was the second scheme evaluated, where the production well is surrounded by a circular battery of small-diameter injection wells. Denitrification conditions are expected to develop along the flow path toward the production well. In this case only one of three injection wells functioned properly. The second one clogged almost immediately after its drilling. The third well penetrated a subformation that was not connected to the production zone. The reduction to nitrate was only 10%, even when the sucrose was consumed completely. The total coliform count was 10 to $10^3$ per 100 ml, so that posttreatment and disinfection were necessary. A very low denitrification efficiency was mainly due to improper function of the injection wells.

In France Boussaid *et al.*[155] proposed *in situ* denitrification using a solid substrate. This system consisted of a production well, three reactors filled with a mixture of straw and calcium carbonate, and three places for reinjection of water into the aquifer through the soil. Cellulose from straw served as an electron donor for heterotrophic denitrification. The effluent from reactors contained organic materials, nitrites, and microorganisms, but it was completely purified on its way through the soil. The installation of prefiltration before reinjection solved the problem with clogging. The nitrate concentration decreased from approximately 60 to 45 mg $l^{-1}$ very quickly in the aquifer. The chemical and microbiological quality of the denitrified water was very good and met drinking water standards without additional treatment.

Braester and Martinell[187] reported on the heterotrophic *in situ* denitrification process Nitredox. In principle, in Nitredox plants injection wells are drilled on the circumference of two rings with different radii around each supply well (*Figure 2*). Injection is performed with simultaneous pumping from the two adjacent wells on the same ring to ensure a good homogenization. A carbonaceous substrate, e.g. methanol, is injected into the outer reduction ring where the nitrate and nitrite are reduced. Water from wells in the inner oxidation ring is pumped via an aerator where nitrogen gas is stripped off and water is saturated with oxygen. Water treated in such a manner is reinjected into the adjacent injection well where a residual content of nitrite is oxidized. To control the *in situ* denitrification, redox electrodes are installed in observation wells located outside the reduction ring, between the reduc-

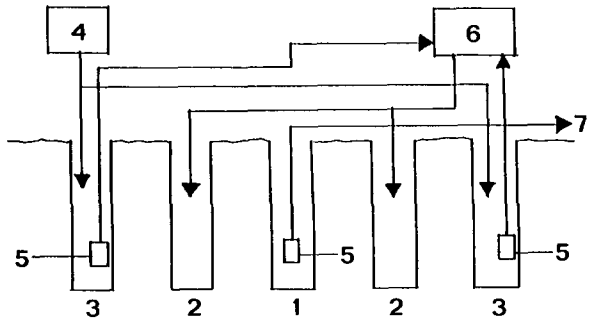


**Figure 2** Layout of wells in a typical Nitredox plant. 1, Production well; 2, oxidation wells; 3, reduction wells; 4, substrate dosage; 5, submersible pump; 6, aeration; 7, denitrified water

tion and oxidation rings, and between the oxidation ring and the supply well.

A plant using the Nitredox process has been constructed in Bisamberg, Austria. The capacity of the supply well is approximately 200 $m^3$ $h^{-1}$. The plant consists of 16 reduction, 8 oxidation, and a number of inspection wells. There are two computerized and fully automatic control systems. The first one serves for the dosage of organic substrate and the other for the control of the oxidation wells. The nitrate concentration is reduced from more than 100 mg $l^{-1}$ to below 25 mg $l^{-1}$ $NO_3^-$. The concentration of nitrite was less than 0.02 mg $l^{-1}$.[187] The microbiological quality of denitrified water has not been reported.

In Germany Selenka *et al.*[188] and Barrenstein *et al.*[158] evaluated *in situ* denitrification using as carbon sources treated wastewaters or methane-rich natural gas. The results of these separate studies showed clearly that the treated wastewater stimulated denitrification activity much better than natural gas. The authors suggested that methane can be used by bacteria only in the presence of oxygen and that a symbiotic relationship may be required for methane utilization for biological denitrification.

The autotrophic *in situ* denitrification process with reduced sulfur compounds has been intensively studied in an unconfined sandy aquifer in Germany.[189–191] A denitrification efficiency of nearly 100% was achieved.

In Switzerland Ginocchio[192] evaluated an autotrophic *in situ* denitrification using hydrogen as an electron donor. The groundwater contaminated with nitrates was withdrawn and amended with hydrogen, carbon dioxide, and phosphate. The amended water was reinjected into the aquifer. The nitrate concentration in the aquifer decreased from 92 to 11 mg $l^{-1}$ $NO_3^-$ within 4 weeks.

*In situ* subterranean denitrification has some problems that are difficult to solve. Denitrification rates in aquifers are rather slow, and it is not easy to control them because they depend, among other parameters, on the dispersion of the aquifer and its geological type. Due to the inhomogeneity and lack of isotropy of aquifers, it is very complicated to ensure homogeneous distribution of substrate. For the process to be effective, it is necessary to use more injection wells. The substrate injection is mostly performed with simultaneous pumping and reinjection of water from adjacent injection wells to increase the homogeneity of substrate distribution in the aquifer. On the other hand, the pumping and recycling of water increases its saturation with oxygen, causing higher substrate consumption and biomass formation in the aquifer. The risk of clogging is very high in this case, even if local clogging of injection wells can be partly reduced by an intermittent injection cycle. The difficulty of controlling a natural bioreactor makes this process applicable only under certain geological conditions.

## Unit processes for biological denitrification

Many different unit processes have been proposed and used for biological denitrification of drinking water.

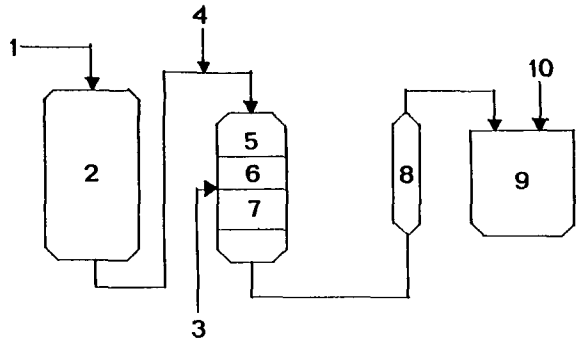


**Figure 3** General layout of Biodenit process. 1, Raw water amended with ethanol and phosphate; 2, anaerobic bioreactor; 3, air; 4, Biocarbone filter; 5, coagulant; 6, activated carbon; 7, sand; 8, ozonation; 9, treated water reservoir; 10, gaseous chlorine and sodium thiosulfate addition

Fixed-film processes, including fluidized-bed reactors, packed-bed reactors, and biofilters are most common. Other processes use immobilized denitrifying bacteria and mixed microbial population in alginate beads or entrapped in a polymer matrix. More innovative fixed-film processes use buoyant porous carriers.

### *Heterotrophic processes*

The most widely used processes for drinking water biological denitrification make use of organic carbon substrates as electron donors. Various solid, liquid, and gaseous carbon sources have been evaluated, such as cellulose, whey, ethanol, biogas, methanol, sucrose, and acetic acid.[146,151,155,193–198]

The first French full-scale potable water denitrification plant was built in Eragny sur Oise and commissioned in June 1983.[199–201] A flow diagram is shown in *Figure 3*. The principle of the Biodenit process is based on a downflow anaerobic bioreactor packed with an expanded clay with grain size between 2 and 5 mm to which heterotrophic bacteria were attached. Ethanol as a carbon source and phosphorus as a nutrient were injected into the raw water.

The 80 $m^3$ $h^{-1}$ facility was designed to treat 1.2 kg $NO_3^-$-N $m^{-3}$ $d^{-1}$. The nitrate concentration was reduced from 68 to 25 mg $l^{-1}$. The subsequent treatment of denitrified water was required. The water was polished on an aerated two-layer activated carbon and sand Biocarbone filter before ozonation and chlorination. Aluminum sulfate was used as a coagulant to help to eliminate the suspended solids. The final concentration of nitrite was maintained at less than 0.05 mg $l^{-1}$.

The operational experience at Eragny had confirmed the economic and technical feasibility of the Biodenit process, which was also employed in the plant in Dennemont near Paris.[200,202] The unit was commissioned at the end of 1987. The capacity of the plant is 400 $m^3$ $h^{-1}$. The nitrate concentration was reduced from 65 to 17 mg $l^{-1}$ $NO_3^-$. The nitrites were not detected in polished denitrified water. The posttreatment was the same as in the Eragny plant.

Another French process, Nitrazur, has been employed in two installations. The first unit utilizing this process was built in 1983 at Chateau Landon.[203,204] The 50 $m^3$ $h^{-1}$ facility used an upflow fixed-bed anoxic bioreactor packed with ceramics. The process consisted of acetic acid and phosphate addition to the raw water, coagulant addition to the denitrified water, carbon filtration, and disinfection using gaseous chlorine. The nitrate concentration was reduced from 80 to 30 mg $l^{-1}$ $NO_3^-$. Nitrite accumulated after the startup but decreased to less than 0.1 mg $l^{-1}$ after steady-state operation was achieved. A similar facility was also built in Champfleur, France.[205] Ethanol was used as a carbon source in this plant.

The technical and economic feasibility of both French processes was confirmed in full scale, and more plants are operating with excellent results. Presently, these methods are economically feasible and the operational stability is very good for a long period.

The German process Denipor is a fixed-bed biological denitrification using buoyant carriers.[21,206–208] This process uses ethanol as a carbon source, phosphate as a nutrient, and sodium hydroxide for pH control. A fixed-bed filter is packed with floating Styropor spheres and utilizes heterotrophic bacteria present in groundwater. The full-scale plant has been constructed at Monheim, Germany. The 300 $m^3$ $h^{-1}$ facility employs four Denipor bioreactors in series. The posttreatment consists of two steps. The first stage employs four aerobic fixed-bed filters with gaseous oxygen addition. The second stage is an aerobic filtration in two fixed-bed filters packed with activated carbon. The addition of chlorine dioxide ensures disinfection of the treated water. The facility is capable of eliminating 80 kg $NO_3^-$-N per day. The nitrate concentration in treated water is well below 10 mg $l^{-1}$. A 95% nitrate removal efficiency was obtained at a loading rate of 1.0 kg $NO_3^-$-N $m^{-3}$ $d^{-1}$. The maximum loading rate of the plant is 1.5 kg $NO_3^-$-N $m^{-3}$ $d^{-1}$. In this case nitrate removal efficiency decreased to 90%.

Holló and Czakó[209] evaluated microbial denitrification using a pilot plant fluidized-bed reactor with effluent recycling to ensure appropriate fluidization. The reactor was 300 mm in diameter and 4 m high. The column was packed with sand and inoculated with pure culture of *Pseudomonas denitrificans*. To enhance the formation of the primary biofilm a cationic polyelectrolyte was used. Propionic acid or ethanol was used as a substrate and phosphoric acid served as a nutrient. Maximum specific nitrate removal rate measured at 12°C was found to be 220 mg $NO_3^-$ $h^{-1}$ $g^{-1}$ cells. The nitrate removal capacity of the reactor was 50 to 60 kg $NO_3^-$ $m^{-3}$ $d^{-1}$ at 10°C. The number of living cells ranged between $10^4$ and $10^6$ $ml^{-1}$ in the effluent from the bioreactor. The subsequent treatment consisted of aeration, sand and active carbon filtration, and chlorination.

Gauntlett and Graft[210] reported a pilot plant operation for biological removal of nitrate from river water. They used a fixed-bed reactor packed with gravel (25 mm in diameter) or a fluidized-bed reactor packed with sand. Methanol was used as a carbon source. The microbial growth was easy to control in the fluidized-bed reactor. The nitrate concentration was reduced by 44 mg $l^{-1}$ at 2°C. A full-scale denitrification unit using this process was constructed in 1982 at Bucklesham, Great Britain, using a chalk spring-fed stream.[9] This plant was the first European full-scale installation using biological denitrification. The denitrification proceeded in an upflow, fluidized sand-bed reactor (1.3 m in diameter, 6.4 m high, sand 0.4 mm in diameter). When the upflow velocity was 20 m $h^{-1}$, the nitrate concentration was reduced from 100 to 25 mg $l^{-1}$. The maximum capacity of the unit was 115 $m^3$ $h^{-1}$. Methanol was added such that a low nitrate concentration was maintained in the effluent to ensure its total utilization. To achieve stable operation of the denitrification process, the addition of 1.5 mg $PO_4^{3-}$ $l^{-1}$ was necessary. The denitrified water required subsequent treatment by coagulation, filtration, aeration, and chlorination. An effective removal was achieved throughout the year, but intermittent high nitrite concentrations were observed.

The use of a fluidized-bed reactor may solve the problems of packed-bed reactors, such as clogging and channeling, which result in an increase of the pressure loss of the bed as well as leading to inhomogeneity problems, often threatening the stable operation of the reactor. Simultaneously, a considerable increase of nitrate removal capacity is achieved. On the other hand, to ensure fluidization of the bed the upflow velocity must be high so that the retention time decreases. The retention is very often too short to achieve a sufficient nitrate elimination. Therefore the recirculation of effluent is used. Fluidized-bed reactors promote process efficiency, but they are much more complicated and difficult to control. Even if promising results have been achieved, there are still a lot of technical problems to be solved.

At the present time, most industrial developments of biological denitrification make use of free microbial cultures and need cumbersome procedures, including coagulation, sedimentation, filtration, and disinfection steps. The use of immobilized organisms is likely to simplify this denitrification treatment, and not surprisingly, several investigations focusing on water denitrification by immobilized denitrifying organisms have already been reported.[146,211–217]

Nilsson *et al.*[212] studied denitrification using *P. denitrificans* cells immobilized in calcium alginate gel. They used potassium asparate as a carbon source. The effects of C:N ratio, pH, temperature, and carbon source on denitrification performance as well as storage and operational stability have been studied. The half-life for nitrate reduction was calculated to be approximately 30 days. The *P. denitrificans* gel retained 75% of its initial activity after storage for 21 days at 4°C.

Nilsson and Ohlson[214,215] reported denitrification in a series of bench-scale columns packed with immobilized *P. denitrificans* cells. The bacteria were encapsulated in alginate gel. Using ethanol as a carbon source, the nitrate concentration was reduced from 104 to 0.1

mg $l^{-1}$ in four columns in the series. The specific denitrification rate was 3.54 g $NO_3^-$ per 1 kg gel (wet weight) per hour. The nitrite concentration in the effluent from the fourth column was 0.3 mg $l^{-1}$. The stability and activity of the gels and cells was relatively short (approximately 2 months). Limitations included the slow transport rate of substrates and metabolites through alginate gels as well as undesirable leakage of cells from the gel matrix.

Kokofuta *et al.*[127,219] used a polyelectrolyte complex consisting of poly(vinyl alcohol) sulfate and poly(diallyldimethylammonium chloride) for immobilization of *P. denitrificans* cells. The activity of immobilized cells was 90% of the free cells at pH 7. The continuous denitrification resulted in 80% reduction of nitrate (from 17.8 to 3.7 mg $l^{-1}$ $NO_3^-$-N) with potassium aspartate as a carbon source. It was found that nitrite accumulated in the effluent only slightly, and its concentration did not exceed 0.04 mg $l^{-1}$ $NO_2^-$-N. After 67 days of operation the percentage of nitrate removal gradually dropped, indicating a fall in the activity of the immobilized system. Lemoine *et al.*[146] used composite structures consisting of a *P. putrefaciens* immobilized cell agar layer bounded by microporous membrane filter for water denitrification. One liter of high-nitrate water (186 $mg^{-1}$ $NO_3^-$-N) with methanol as a carbon source was completely freed from nitrate and nitrite in 11 days at a rate of 90 mmol $NO_3^-$-N $h^{-1}$ $g^{-1}$ of agar gel. When acetic acid was used instead of methanol, the rate decreased to 30 mmol $NO_3^-$-N $h^{-1}$ $g^{-1}$ of agar gel. The membrane filter prevented any contamination of the treated water. The identical system was used by Mignot *et al.*[216] The composite structure containing *P. denitrificans* and operated at low C : N (2 : 1) ratio developed the highest denitrifying activity. The average denitrification rate was 0.22 mg $NO_3^-$-N $h^{-1}$ $g^{-1}$ of agar gel. It was lower than that reported by Nilsson *et al.*[217] for *P. denitrificans* cells immobilized in alginate beads. This difference is probably due to a larger active surface of beads per unit weight of gel and increased diffusion problems brought about by the microporous membrane. However, the slower denitrification rate was balanced by the quality of treated water where microbial contamination was absent.

Čižinská *et al.*[213] and Maixner *et al.*[219] reported a preparation of cell aggregates with denitrification activity. They used a mixed population of activated sludge immobilized in water-soluble polymer formed by reaction of branched polyethyleneimine and glutaraldehyde. The biocatalyst was used in a pilot-plant study.[220] The expanded-bed reactor (100 mm in diameter, 1000 mm high) was packed with 2 kg of biocatalyst (wet weight). The dry mass content of the biocatalyst was 30% w/w. The column was fed with groundwater (nitrate content from 78.0 to 91.3 mg $l^{-1}$) supplemented with ethanol. Continuous denitrification was performed more than 100 days. The average specific denitrification rate was 2.31 mg $NO_3^-$ $h^{-1}$ $g^{-1}$ of dry mass of biocatalyst. The half-life of the biocatalyst was calculated to be more than 3 months. The quality of denitrified water was such that posttreatment and disinfection was required to match quality standards for drinking water. It was proved that microbial contamination was due to the washout of microorganisms from the surface of the biocatalyst. It was not caused by leakage of microorganisms from the biocatalyst.

The use of immobilized cells could lessen the contamination of denitrified water by microorganisms, but it cannot solve the problem completely. When the system is operated continuously, it is not possible to prevent formation of a biofilm on particles of immobilized cells during operation. The washout of microorganisms occurs sooner or later, so that the denitrified water is contaminated. Postreatment has to be employed to match the water quality standards. It is questionable if the use of immobilized cells for denitrification could be economically feasible due to relatively high costs of immobilization and limited operational time of the biocatalyst.

The only method that is competitive with biological denitrification is the ion exchange method. Its serious disadvantage is the production of a voluminous brine during regeneration of a resin. This brine contains very high chloride, bicarbonate, sulfate, and nitrate concentrations, and hence it is very difficult to dispose of. This problem was partially solved by van der Hoek and his coworkers using combined ion exchange/biological denitrification.[11,221-225] In this process the regenerant, containing the nitrate removed from the ion exchanger, is treated by the biological denitrification reactor. A demonstration plant has been built which consists of three ion exchange columns, an upflow sludge blanket denitrification reactor with a working volume of 3.3 $m^3$, and a sand filter in the regeneration circuit. The sand filter is used to remove suspended solids washed out from the denitrification reactor. In this way the contamination of the resin with sludge particles can be avoided. An average nitrate concentration in the raw water was 61 mg $l^{-1}$. Using a flow rate of 11 $m^3$ $h^{-1}$, 90% nitrate removal was achieved. The combined ion exchange/biological denitrification process resulted in a reduction of 40 to 80% in brine volume.[221] The process is attractive as compared with conventional ion exchange processes, but problems were met concerning a stable operation of the upflow sludge blanket denitrification reactor. Due to varying nitrate load of the bioreactor, nitrite accumulation occurred. A limitation for application of the process is a considerable chloride concentration increase in treated water. In contrast with biological denitrification, no extensive posttreatment of the denitrified water is required.

### *Autotrophic denitrification*

Many schemes for autotrophic denitrification have been proposed. Investigators have used various substrates such as hydrogen or reduced sulfur compounds. Both fixed-bed and fluidized-bed reactors have been used in studies of denitrification kinetics, stoichiometry, and efficiency.

Kurt *et al.*[166] studied autotrophic denitrification in a fluidized sand-bed reactor using a mixed culture and hydrogen. They used a cone-shaped reactor which caused a reduction of linear flow rate from bottom to top. This velocity gradient resulted in a density gradient of biofilm-sand particles with the largest biofilm particles at the top and relatively clean sand at the bottom. Batch experiments always exhibited nitrite accumulation, but continuous experiments in a bench-scale system resulted in complete nitrogen removal provided that residence time was sufficient. For complete denitrification of water containing 25 mg $l^{-1}$ $NO_3^-$-N, a residence time of 4.5 h was required. The optimum pH was found to be 7.5. If the pH was allowed to rise to 9.0, nitrite tended to accumulate. Rates of up to 23 mg $l^{-1}$ $h^{-1}$ $NO_3^-$-N were achieved. The dynamic effects of flow variation on steady-state operation observed during continuous operation indicated that the process could best be operated as a multistage process.

Various authors[22,171,226-231] have reported on the development and performance of a commercial-scale biological denitrification plant utilizing hydrogen and a mixed autotrophic culture at works Rasseln near Monchengladbach, Germany. The technology was developed by Sulzer A. G., Winterthur, Switzerland and given the trade name Denitropur. The startup began in early 1986 and reached full capacity in approximately 3 months.

The plant consists of nine fixed-bed denitrification bioreactors in a series packed with Mellapack, mixing elements with a three-dimensional corrugated structure. The raw water is saturated with hydrogen under overpressure and enriched with phosphate and carbon dioxide. After denitrification the water is aerated and filtered on a two-layer filter. Disinfection is ensured by means of UV radiation. The 50 $m^3$ $h^{-1}$ facility eliminated nitrate concentration from 75 to less than 5 mg $l^{-1}$ $NO_3^-$ within a residence time of water in the reactors of about 1 h. The plant is capable of removing 90 kg $NO_3^-$ per day. The process is schematically shown in *Figure 4*.

The kinetics of autotrophic denitrification using hydrogen in a fluidized-bed reactor have been studied by Tuisel *et al.*[232] The reactor was packed with sand. In bench-scale experiments, the $NO_3^-$ content of raw water, 131.5 mg $l^{-1}$ was reduced to 0 mg $l^{-1}$ after 5 h. The pH was controlled at 7.0 by acid addition. When phosphate was not added to the raw water, the denitrification efficiency decreased by nearly 70%. The operational temperature decrease from 18 to 12°C reduced the denitrification rate by 33%. The maximum denitrification rate at 18°C was 2.24 kg $NO_3^-$ $m^{-3}$ $d^{-1}$.

Based on extensive research with laboratory-scale and pilot-scale experiments,[233-235] a demonstrated plant using sulfur/limestone filtration was built in the Netherlands.[221] The process scheme is depicted in *Figure 5*. The plant consisted of four unit operations in series: vacuum deaeration of the raw water, upflow filtration in a bioreactor packed with sulfur and limestone, aeration, and artificial recharge. Vacuum deaeration was employed to prevent supersaturation of the water with nitrogen gas resulting in clogging and channeling of the bioreactor. The 35 $m^3$ $h^{-1}$ plant used a reactor (20 m long, 7 m wide, 2 m deep) filled with sulfur granules and limestone granules in 1 : 1 volume ratio. The raw water contained 70 to 80 mg $l^{-1}$ $NO_3^-$. During a period of 200 days, nitrate removal exceeded 90%. The denitrified water from the sulfur/limestone bed contained high numbers of bacteria, up to $10^6$–$10^7$ cells $ml^{-1}$, and very high concentrations of assimilable organic carbon. However, artificial recharge and withdrawal after 10 to 12 weeks' residence time resulted in an almost complete reduction of the colony counts and provided water of excellent bacteriological quality.

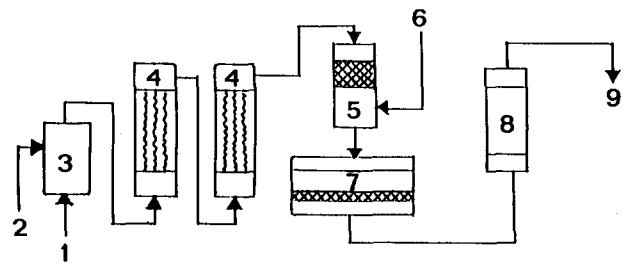


**Figure 4** Scheme of Denitropur plant. 1, Raw water; 2, hydrogen; 3, hydrogen saturation; 4, bioreactor; 5, countercurrent aerator; 6, air; 7, two-layer filter; 8, UV disinfection; 9, denitrified water

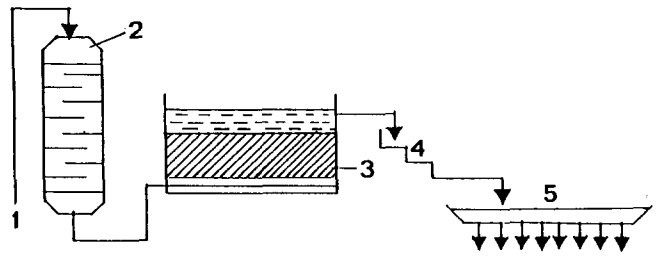


**Figure 5** Process scheme of a sulfur/limestone denitrification plant. 1, Raw water; 2, vacuum deaerator; 3, sulfur/limestone denitrification reactor; 4, cascade; 5, infiltration pond

Le Cloirec and Martin[83] and Le Cloirec *et al.*[84] developed a mathematical model for denitrification kinetics by *Thiobacillus denitrificans* in an upflow reactor filled with sulfur/calcium carbonate. The model considered biomass growth, nitrate removal, nitrite evolution, and consumption of sulfur. The influence of the nitrate load and hydraulic load on the treated water was studied. A good correlation was obtained between experiments and simulated values.

The bench-scale experiments were performed with raw water containing 50 to 100 mg $l^{-1}$ $NO_3^-$, 250 mg $l^{-1}$ carbonate, and 0.5 mg $l^{-1}$ $PO_4^{-3}$. The denitrification rate was up to 4.8 kg $NO_3^-$ $m^{-3}$ $d^{-1}$.

The problem connected with the use of reduced sulfur compounds is the evolution of sulfate. Denitrification of wastewaters containing high nitrate concentrations can result in sulfate inhibition of the process due to high concentrations of sulfate produced in treat-

**Table 4** Performance of selected biological nitrate removal technologies

| Electron donor | Carrier | Bed type | Removal rate (kg $NO_3^-$-N $m^{-3}$ $d^{-1}$) | Temperature (°C) | Flow ($m^3$ $h^{-3}$) | Ref. |
|---|---|---|---|---|---|---|
| *Autotrophic denitrification* | | | | | | |
| Hydrogen | Sand | Fluidized | 0.50 | 18 | – | 232 |
| | | | 0.32 | 12 | – | 232 |
| | | | 0.55 | – | – | 166 |
| Hydrogen | Plastic | Fixed | 0.25 | 10 | 50 | 226 |
| Hydrogen | Polyurethane | Fluidized | 1.30 | 20 | – | 236 |
| | | | 0.60 | 5 | – | 236 |
| Sulfur | $CaCO_3$ | Fixed | 1.08 | 15 | – | 83 |
| | | | 0.30 | 15 | 35 | 84 |
| | | | 0.14 | – | – | 237 |
| *Heterotrophic denitrification* | | | | | | |
| Methanol | Sludge blanket | | 7.20 | – | 14 | 238 |
| Methanol | Sand | Upflow filter | 2.70 | 21 | 11 | 239 |
| Sodium acetate | Plastic | Fluidized | 2.10 | – | – | 240 |
| Acetic acid | Clay | Fixed | 0.40 | 10 | 200 | 197 |
| Sugar | Silicate | Fixed | 1.15 | 12 | 20 | 241 |
| Propionic acid | Sand | Fluidized | 11.30 | 10 | – | 209 |
| Potassium aspartate | Polyelectrolyte complex | Fixed | 260.00[a] | 21 | – | 218 |
| Ethanol | Expanded clay | Fixed | 1.20 | 12 | 400 | 198 |
| Ethanol | Alginate gel | Fixed | 24.00[a] | 22 | – | 215 |
| Ethanol | Polystyrene | Fixed | 1.50 | 10 | 300 | 206 |
| Ethanol | Sand | Fluidized | 2.60 | 11 | – | 242 |
| Ethanol | Polymer aggregates | Expanded | 12.10[a] | 12 | – | 220 |
| Ethanol | Agar gel | Fixed | 1.20[a] | – | – | 216 |

[a] Data in g $NO_3^-$-N per kg of biological material per day

ment. This is not the case with potable water denitrification because nitrate concentrations to be treated are lower and sulfate inhibition would not occur. On the other hand, the potability of water may be reduced due to higher sulfate concentrations. Reduction of 152 mg $l^{-1}$ $NO_3^-$ using elemental sulfur would yield stoichiometrically 250 mg $l^{-1}$ sulfate, which is the upper limit in most water quality standards.

## Conclusions

Nitrate contamination of drinking water sources is an ever-increasing problem. That is why a significant amount of research has been done regarding denitrification.

*In situ* denitrification is a very attractive alternative due to relative simplicity and low investment and operational costs. On the other hand, there are a lot of problems in maintaining a stable operation, achieving homogeneous substrate distribution, and retaining a sufficient denitrification rate if autotrophic denitrification is used. The risk of clogging due to biomass accumulation and gas production is very high. The quality of the denitrified water might require posttreatment and disinfection, which increase overall treatment costs. It is very difficult to control biological processes during *in situ* denitrification. Generally, sites for *in situ* biological denitrification should be carefully selected because this process is applicable only under certain geological conditions.

Various unit processes have been adapted and used for heterotrophic or autotrophic denitrification of drinking water. The performance of selected processes is listed in *Table 4*. It can be seen that heterotrophic denitrification processes possess much higher specific volumetric denitrification rates than autotrophic ones.

Autotrophic processes employ mostly hydrogen as a substrate for denitrification. In this case the production of biomass is very low and only minimal amounts of excess biomass need to be disposed of. Clogging problems due to biomass formation are minimal in fixed-bed reactors. On the other hand, the reaction rate of autotrophic bacteria is very low, so that bioreactors with large volumes are required to achieve sufficient residence time for efficient denitrification. The other limitation is very low solubility of hydrogen in water, so that intensive recirculation or operation under pressure is necessary. These disadvantages result in higher investment costs as well as operating costs in comparison with heterotrophic denitrification.

Heterotrophic denitrification is a well-known process. This technology is now applied in several full-scale installations using fixed-film systems which differ in support materials, substrate used, and back-washing techniques. The technical and economic feasibility was confirmed in full-scale operations in France, Great Britain, and Germany. Most commercial applications use fixed-bed bioreactors because of the relative operational simplicity and the ease of process control.

Even though significant progress has been made in research on biological denitrification, more research is needed. Areas that require further study include the minimization of nitrite accumulation, optimization of reaction conditions, inhibitory effects of reaction in-

termediates and by-products, and optimization of measuring and control systems.

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