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Transformations of ammonia and the environmental impact of nitrifying bacteria

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Abstract

In the sequence of events leading from ammonia to N_2 during the process of biotransformation of inorganic nitrogen compounds, the weakest link, with respect to our knowledge and understanding of the organisms involved, is nitrification. In particular, this is true for the oxidation of ammonia to nitrite. The enzymes have not been thoroughly studied, and the enzymatic mechanisms have not been identified. Almost any biochemical and physiological aspect studied proved to be controversial, and major ecological questions still remain unanswered. Unless the structure and function of the various components of the process are worked out, progress in developing means for controlling nitrification will depend mainly on laborious trial and error and not on knowledgeable manipulation of this group of bacteria.

Abbreviations: AMO – ammonia monooxygenase; HAO – hydroxylamine oxidoreductase; MPN – most probable number; TCE – trichloroethylene

General aspects

All major transformations of inorganic nitrogen in the environment, such as nitrogen assimilation, nitrification and denitrification are carried out exclusively by microorganisms. While considerable research effort is put into attempts to increase nitrogen assimilation for agricultural purposes, much less is invested in studies on the dissimilatory processes, particularly natural nitrification. This activity is carried out by a small group of bacteria which, in spite of their key role in global nitrogen cycling, is still poorly understood.

Environmental pollution by inorganic nitrogen is the result of a disequilibrium between input of fixed nitrogen (biotic and abiotic), usually in the form of ammonia, and its output, usually in the form of N_2, the only form of nitrogen which can be considered as environmentally safe. All other forms of nitrogen, which can accumulate due to the establishment of unbalanced fluxes, may create problems ranging in their severity from mild nuisances to serious ecological hazards.

The global biogenic fluxes of inorganic nitrogen are governed by 3 major processes: nitrogen fixation, nitrification and denitrification. Annual global input of fixed nitrogen (natural and industrial) is estimated at $1.75 \cdot 10^8$ tons (Bock et al. 1989). Although it may exist in a global equilibrium with natural denitrification, on smaller scales we find greatly unbalanced fluxes of inorganic nitrogen due to ever increasing anthropogenic activities. Only by manipulating and maintaining environmental factors to allow microorganisms to transform fixed nitrogen to dinitrogen gas, can we avoid the consequences of the accumulation of biologically active

inorganic nitrogen species. Success depends on an in depth understanding of the processes involved, at all levels, from biochemistry to ecology, as with any other biological activity.

Pollution of the environment with nitrogen differs from pollution by other biogenic pollutants such as carbon and phosphorous, because not only can it trigger eutrophication of water bodies or affect soils and atmosphere, but some forms (ammonia, NO_2^-, NO_3^-) are, to varying degrees, toxic to various life forms, depending on the organisms exposed and upon environmental factors such as pH and temperature.

When compared to other environmentally important biogenic inorganic ions (PO_4^{3-}, Fe^{3+}, S^{2-}, CO_3^{2-}, SO_4^{2-} and others) the major nitrogenous end products of degradation of organic matter also differ in that all forms of inorganic nitrogen except for dinitrogen are very soluble, and can therefore reach high concentrations.

Of the various forms of nitrogen, ammonia and nitrite are the most toxic to aquatic wildlife (fish, crustaceans, mollusks). Concentrations of 0.1–1.0 mg NH_3^- N·l^{-1} are usually considered lethal (Kinne 1976). Because biological membranes are relatively impermeable to the ammonium ion, but readily permeable to unionized ammonia, toxicity of ammonia is a function of the pH. Nitrite concentrations should not exceed 0.1 mg·l^{-1} in closed water systems (King & Spotte 1974). Nitrate is less dangerous because it is toxic at much higher concentrations: 250–350 mg·l^{-1} nitrate were found to be lethal to many fish (DeGraff 1964). The US standard for nitrates in drinking water limits its concentration to 45 mg·l^{-1} (USEPA 1976), while European standard is 50 mg·l^{-1} (EEC 1980).

The maximum contaminant level allowed by recent EPA regulations for nitrite in drinking water is 1.0 mg·l^{-1} (Pontius 1991). It is therefore obvious that for all practical reasons nitrogen assimilation and dissimilatory denitrification must be optimally balanced. If not, either excess assimilation will cause accumulation of toxic inorganic nitrogen, or, on the other hand, denitrification might reduce primary productivity, affecting agriculture and the capacity of any ecosystem to sustain life.

Main inputs of ammonia and nitrite into the environment are through microbial nitrogen fixation and anthropogenic sources such as wastewater discharge and the widespread use of industrial fertilizers. The main sources of NO_x originate from microbial nitrification and denitrification processes, as well as from various industrial and urban activities.

Comprehensive reviews have been recently published on nitrogen fixation (e.g. Gresshoff et al. 1990) and on microbial denitrification (e.g. Revsbech & Sorensen 1990). Therefore, this review focuses primarily on nitrification and nitrifying bacteria, with an emphasis on the group of chemolithotrophic bacteria oxidizing ammonia to nitrite. Although they play a key role in cycling of inorganic nitrogen in the environment, they are the least studied group involved in this process. The reasons are that ammonia oxidizing bacteria are difficult to cultivate, and the enzymes involved in the oxidation of ammonia are difficult to work with. The information presently available is sketchy, incomplete, and subject to controversy.

Nitrification

Although nitrification is a general term used conventionally to describe the oxidation of reduced nitrogenous compounds by chemolithotrophic bacteria, as sole or primary energy source, it is by no means a sole oxidative pathway. Heterotrophic nitrification, not coupled to any energy yielding metabolic pathways, has been demonstrated in many organisms, including bacteria, such as *Arthrobacter* sp. (Witzel & Overback 1979), *Alcaligenes faecalis* (Papen et al. 1989), *Thiosphaera pantotropha* (Robertson & Kuenen 1988), and fungi (for a review see Killham 1986). Even mammals have been shown to be nitrifiers (Green et al. 1985). A possible mechanism for the accumulation of nitrite by heterotrophic organisms is the cooxidation of ammonia to nitrite, coupled to the xanthine oxidase reaction (Nagano & Fridovich 1985).

Chemolithotrophic nitrification is a two stage oxidation of reduced inorganic nitrogen to nitrite and then to nitrate. Different groups of chemo-

lithotrophic bacteria carry out the 2 reactions, and no single known bacterium is capable of complete oxidation of ammonia to nitrate in a single step.

Enzymes of nitrification

The first step in nitrification is the oxidation of ammonia to nitrite, best studied in various species of the genus *Nitrosomonas*. Two enzymes catalyze two key reactions:

$$NH_3 + 2H^+ + 2e^- + O_2 \rightarrow NH_2OH + H_2O$$
(ammonia monooxygenase)

$$NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^-$$
(hydroxylamine oxidoreductase)

Only the second reaction provides ammonia oxidizing bacteria with their energy requirements.

The second step is the oxidation of nitrite to nitrate:

$$HNO_2 + H_2O \rightarrow HNO_3 + 2H^+ + 2e^-$$
(nitrite dehydrogenase)

carried out by members of the *Nitrobacter* and *Nitrospira* genera.

The first two key enzymes involved in these reactions proved to be difficult to work with. Ammonia monooxygenase (AMO) is a membrane bound protein. It has not been isolated, and all the information presently available regarding its structure and mode of action is therefore purely circumstantial. Hymen & Wood (1985) showed that acetylene irreversibly inactivates the oxidation of ammonia, and in a crude extract of *N. europaea* they also showed the presence of a single 28 kd protein which reacts with acetylene. This is, at present, the only clue to the identification of the protein responsible for the AMO activity. Hydroxylamine oxidoreductase (HAO) is a soluble 180,000–200,000 kd periplasmic protein. The present state of information with regard to its structure and mode of action leaves much to be desired (Hooper et al. 1978; Yamanaka et al. 1979; Terry & Hooper 1981; Olson & Hooper 1983; Hooper 1984; Hooper et al. 1984; Hooper et al. 1990). The situation with regard to nitrite dehydrogenase is better, as the enzyme was purified and described in detail by Yamanaka & Fukumori (1988).

Oxidation of ammonia to nitrite requires one atom of oxygen derived from O_2 (Hollocher et al. 1981), while the source of the second atom of oxygen is water (Andersson & Hooper 1983). Water is also the source of the oxygen incorporated into nitrate during the oxidation of nitrite by *Nitrobacter* (Aleem et al. 1965).

Habitats of nitrifying bacteria

As true chemolithotrophic microorganisms, nutritional requirements of nitrifiers are minimal, and in practice, in any aerobic ecological niche where traces of ammonia are present, nitrifiers will also be found. Thus they penetrate rocks and sandstone of historical monuments to the depth, causing acidification and deterioration (Bock 1989). Likewise, nitrifiers have been found responsible for destroying asbestos-cement roofs of buildings (Wasserbauer et al. 1988). Nitrifiers have been isolated, and nitrifying activity has been described in Antarctic sea ice (Priscu et al. 1990). They have been found responsible for the depletion of oxygen under Arctic winter ice (Knowles & Lean 1987). Nitrifiers have also been implicated in soil acidification (Van Miegroet & Cole 1984, 1985). The impact of nitrifiers can be significant even when pollution sources are remote and contact indirect. For instance, although there is very little human activity on the shores of Lake Superior, its levels of nitrates increased 4-fold during the past century, primarily due to loading of nitrogenous compounds from the atmosphere (Bennet 1986). Nitrifiers also cause specific problems in disinfecting facilities for drinking water. When nitrite accumulates, due to incomplete nitrification or denitrification, it reacts readily with chlorine, decreasing effective residual chlorine concentrations. It also interferes with chloramination (Wolf et al. 1988). Heavy agricultural fertilization frequently results in massive nitrification and leaching of nitrates to ground water,

increasing concentrations of nitrates to levels beyond permitted standards (Soares et al. 1991).

Nitrification as a source for nitric and nitrous oxides

NO_x gases play an important role in the stratospheric ozone cycle and appear to be much more effective green house gases than CO_2. Their reaction with volatile organic carbon also contributes to tropospheric ozone accumulation. However, the significance of nitrifiers as NO_x producers is not clear. Ammonia oxidizing bacteria are known to be capable of reducing nitrite to NO and N_2O (Hooper 1968). Ritchie & Nicholas (1972) attributed part of the NO_x produced aerobically to direct oxidation of NH_2OH. Later studies did not resolve the question whether NO_x are released by nitrifiers as a product of a denitrification reaction under microaerophilic or anaerobic conditions, or also by direct oxidation of ammonia. Although Poth & Focht (1985) stated that *Nitrosomonas* releases N_2O only as a denitrifying reaction, Hooper et al. (1990) also showed that some of the N_2O may be produced through oxidation of ammonia. Also, the data presented by Kim & Craig (1990) suggested that nitrification is the origin of some N_2O in deep ocean water. A possible mechanism of accumulation of NO and N_2O during nitrification by *Nitrosomonas* is that under certain conditions (e.g. the presence of organic reductants) excess hydroxylamine is released by the cells and reacts chemically with nitrite to form NO and N_2O (E. Bock, pers. comm.). There is also a controversy as to the contribution of nitrifiers vs. heterotrophic denitrifiers to total NO_x produced in natural habitats. While the data presented by Klemendtsson et al. (1988) and Downes (1988) suggested that nitrifiers play a major role in N_2O production in soils and freshwater lakes, according to Yoshida (1988) the role of nitrifiers in the production of N_2O in the oceans has been overestimated. Interestingly, Remde & Conrad (1990) showed that NO is produced by ammonia oxidizers and that in contrast to the release of N_2O, this production of NO is insensitive to oxygen concentration.

Biodegradation of hydrocarbons

While early attempts to demonstrate oxidation of methane, methanol and CO by *Nitrosomonas* failed (Drozd 1976), it has since been demonstrated that hydrocarbons are metabolized by a wide range of chemolithotrophic ammonia oxidizers (Hyman & Wood 1983; Jones & Morita 1983; Voysey & Wood 1987; and see Bedard & Knowels 1989 for a review), apparently due to the nonspecificity of ammonia monoxygenase. Although attempts to grow ammonia oxidizers with methane as a sole energy source failed, methane was completely oxidized to CO_2. On the other hand, some methanotrophs are capable of oxidation of ammonia to nitrite (Hutton & ZoBell 1953; Wittenbury et al. 1970). Other hydrocarbons are also co-metabolized by ammonia oxidizers. Acetylene is a suicidal substrate (Hyman & Wood 1985) to *Nitrosomonas*, apparently due to irreversible covalent bonding to the AMO. Ethylene is oxidized by *N. europaea* to ethylene oxide, a reaction sensitive to inhibitors of ammonia oxidation. Ethylene oxide is also further metabolized (Hyman & Wood 1984). Hyman et al. (1988) also demonstrated that n-alkanes (C_1–C_8) were oxidized to their respective alcohols, with increasing rates from C_1 to C_4 and a marked decreased rate above C_4. This oxidation depends on the presence of ammonia as a reductant for the AMO reaction, or, to a limited extent, on endogenous reducing power. All these oxidations were inhibited by AMO inhibitors (acetylene and thiourea) except for some residual activity when 1-pentene was tested. On the other hand, alkynes (C_2–C_{10}) were inhibitory to ammonia but not to hydrazine oxidation. Halogenated hydrocarbons are also degraded by ammonia oxidizers. Vannelli et al. (1990) demonstrated ammonia dependent, AMO mediated, oxidation of a range of halogenated hydrocarbons by *N. europaea*, including dichloromethane, dibromomethane, trichloromethane, cis and trans, dichloro-and dibromo-ethylene, trichloroethylene and trichloropropane (but not tetrachloroethylene) without the inactivation of the enzyme. AMO inhibitors inhibited these reactions by at least 70%. The halogenated hydrocarbons competed with ammonia for the AMO active site. Like-

wise, Rasche et al. (1990a) demonstrated that all haloethanes are oxidized (F, Cl, Br, I) to acetaldehyde, and that all tested n-chlorinated alkanes (C_1–C_4) were also oxidized to the corresponding aldehydes. Soil nitrifiers (*Nitrosomonas europaea* and *Nitrosolobus multiformis*) degraded nematicidic soil fumigants (methyl bromide, 1,2,dichloropropane, 1,2,dibromo-3-chloropropane) (Rasche et al. 1990b). These degradations were also inhibited by AMO inhibitors such as acetylene and allylthiourea. However, Rasche et al. (1991) demonstrated, using *N. europaea* and trichloroethylene (TCE), that active protein synthesis during the reaction is required to prevent irreversible inactivation of AMO by TCE. Other chlorocarbons varied with respect to their effects of AMO activity.

Aromatic hydrocarbons are also oxidized by the AMO of *N. europaea*. Hymen et al. (1985) showed that benzene is oxidized first to phenol, which was further oxidized to hydroquinone, but not to catochol or resorcinol.

Environmental studies

As mentioned above, oxidation of ammonia to nitrite/nitrate is a key reaction in nitrogen cycling, because there is no biological reciprocal of the nitrogen fixation reaction, back from ammonia to dinitrogen. The majority of nitrogen fertilizer added in agricultural practice is in the form of ammonia, which is adsorbed by the soil particles and then slowly released. However, in the presence of nitrifiers, ammonia is also rapidly oxidized to nitrate which is then lost either through leaching to groundwater (polluting aquifers) or to surface waters (polluting lakes and rivers) or it is oxidised to nitrous and nitric oxides (see above). Under anaerobic conditions, if electron donors are available in sufficient concentrations, nitrates may be lost by their reduction to dinitrogen. Therefore, direct measurements of nitrification potential, number of nitrifiers, and actual nitrification rates, in agricultural lands, as well as in forest ecosystems, submerged soils, and heath soils are needed for accurate estimates of nitrogen fluxes through these habitats.

Many difficulties arise when attempts are made to correlate laboratory studies to actual field conditions, some of them due to two practical factors. Their growth rates are very slow in liquid media, and they cannot be counted by plating on solid media. Therefore the numbers of viable and culturable nitrifying organisms can be estimated only by the most probable number method (MPN), being then followed by measuring concentrations of nitrite and nitrate in the dilution series. Their slow growth rate prolongs incubations. The most common incubation period for MPN counts is 3 weeks, although Matulewich et al. (1975) showed that 8 weeks incubations yield much more accurate results; and Hashimoto & Hattori (1987) suggested even longer incubation periods. Therefore, counts of nitrifiers can serve only as minimal estimates of their true concentrations. Other parameters used, such as measuring nitrification potential, are also questionable, this because of the difficulties in simulating *in situ* conditions in the laboratory. For example, Berg & Rosswall (1985) reported that in their study on arable soils in Sweden, actual nitrification rates were 5–25 times lower than potential rates determined in the laboratory.

Inhibition of nitrification

Phenolic acids excreted by vegetation were cited as inhibitors of nitrification (Rice & Pancholy 1972). On the other hand, McCarty et al. (1991) found no basis for the assumption that phenolic acids inhibit ammonia oxidation in terrestrial ecosystems. Others claim that fulvic and humic acids enhance oxidation of ammonia to nitrite, but inhibit its further oxidation to nitrate (Tan & Lopez-Falcon 1987a, 1987b). Low pH, as well as organic matter, are frequently cited as factors that limit nitrification in forest ecosystems (White & Gosz 1987). However, although nitrification is considered to be pH sensitive, there are several reports describing autotrophic nitrification in acidic environments such as an acid heath soil (pH 3.6–4.0) (DeBoer et al. 1988), as well as ureolytic autotrophic nitrification by *Nitrospira* sp. at pH 4.8 (DeBoer 1989). Hankinson & Schmidt (1988) have also isolated an

acidophilic *Nitrobacter* from acid forest land. Although nitrification is also considered to be sensitive to excess ammonia and nitrite, Blouin et al. (1989) reported a complete oxidation of very high concentrations of ammonia nitrogen (1,000 $mg \cdot l^{-1}$) in swine wastes in 5 days to nitrite, and another 10 days were needed for oxidation of all nitrite to nitrate.

In spite of their ubiquitous distribution, nitrifiers do suffer from various unidentified inhibitors, and the addition of powdered activated carbon to activated sludge wastewater treatment process enhances nitrification by adsorbing inhibitory compounds (Ng & Stenstrom 1987). The issue of protection of nitrifiers from various inhibitors by their attachment to surfaces is also a subject of controversy. While there are claims that surface attachment appears to protect nitrifying bacteria from a range of inhibitors (Ratnayake & Audas 1978), others have shown that surface growth alone does not protect soil nitrifiers from the effect of inhibitors (Underhill & Prosser 1987).

Another factor believed to have a detrimental effect upon nitrification is light. Hooper & Terry (1973, 1974) have shown that visible light (420 nm) inhibits oxidation of ammonia but not of hydroxylamine, at a rate constant proportional to its intensity. Inhibition by light also abolishes the ability of the putative AMO peptide to bind acetylene (Hyman & Arp 1992). U.V. light inactivated both oxidation of ammonia and NH_2OH. Protection against photoinactivation could be achieved either by fully inhibiting the nitrification process, as by anaerobiosis, or, conversely, by allowing rapid oxidation of ammonia during illumination. Similarly, Shears & Wood (1985) reported that protection against photoinactivation could be provided by ammonia monooxygenase substrates as well as by anaerobiosis. While Hooper & Terry (1974) reported that recovery of ammonia oxidation by photoinhibited cells took 6 hours, others reported very different recovery rates, up to 120–350 days (Yoshioka & Saijo 1984). From various reports it seems that photoinhibition is not a universal phenomenon. There have been reports on variable or adaptive responses (Horrigan & Springer 1990), while others did not find any inhibitive effect of light (Ward 1984) or even report on light stimulation of ammonia oxidation (Miyazaki et al. 1973). Starvation of *Nitrosomonas cryotolerans* also seemed to sensitize them to photoinhibition (Johnstone & Jones 1988). However, as will be described in the following section, nitrifying bacteria have frequently been found in ecosystems that are permanently exposed to direct sunlight.

Ecophysiology of nitrifying bacteria

In a world where many suffer from food shortages due to poor agricultural practices and insufficient fertilization, where efforts are made to increase microbial nitrogen fixation and to transform staple food plants genetically to become nitrogen fixers as well, the question of inorganic nitrogen pollution seems somewhat out of place. Nevertheless, inorganic nitrogen pollution is a real problem in many parts of the world. It originates either from excess nitrogen fertilization, or as a product of catabolic deamination, releasing ammonia into the environment. This is either through biodegradative processes, or as part of intensive treatment of domestic sewage or agricultural wastes. It is these anthropogenic sources of discharged ammonia which cause a wide range of environmental problems.

The sequence of events leading to the elimination of excess nitrogen from the environment is a complex string of reactions performed by a consortium of microorganisms. For example, during the treatment of domestic wastewater, microbial mineralization of organic matter ends with large quantities of ammonia (80–120 mg/l) in the effluents, and unless a nitrification-denitrification stage is added to the process, these are discharged as such to the environment. When climatic conditions permit and land is available, oxidation ponds are the method of choice for treatment of sewage, due to their low operational costs and high efficiency in eliminating pathogens. Here, ammonia plays a cardinal role in reducing the efficiency of the process. It has been long known that ammonia uncouples electron flow in isolated chloroplasts (Avron & Shavit 1965) and inhibits the whole photosynthetic process in intact algal cells (Abeliovich & Azov

1976). For example, 2–3 mM ammonia at pH 8.1–8.4, will cause 50–90% inhibition in the rate of O_2 generation by any algal population present in the ponds. This phenomenon has far reaching consequences. Efficient and nuisance free operation of oxidation ponds calls for uninterrupted and optimal oxygenation of the water. This is done almost exclusively by the algal population which is expected to dominate the pond. However, un-ionized ammonia penetrates freely through the cell membrane and, unless kept below ca 2 mM, reaches intracellular concentrations which strongly inhibit photosynthesis and oxygenation in a pH dependent manner. Uninterrupted photosynthesis can, in eutrophic water bodies, elevate pH to above 10, but, in the presence of ammonia, it will elevate the pH only to that level at which unionized ammonia will reach intracellular concentrations inhibitory to photosynthesis. Theoretically, respiration can lower the pH to levels at which the concentration of unionized ammonia will not be inhibitory to photosynthesis. But, for respiration to lower pH, oxygen is needed. This vicious circle can be interrupted only by enhancing nitrification (Abeliovich 1983).

Nitrification of the ammonia in domestic sewage is easily achieved in the laboratory in experiments aimed at simulating oxidation pond or wastewater reservoir conditions (Abeliovich 1985). But nitrification in real oxidation ponds is a very slow process (Abeliovich 1987), with measured rates usually within the range of 0.5–1% of the ammonia being oxidized every day.

The reasons for this slow rate of nitrification are not clear. Light, although effective at inhibiting nitrification in mineral medium in the laboratory, had variable effects when tested with wastewater reservoir effluents, and had no effect upon viability of nitrifiers in the field (Abeliovich 1987). In wastewater reservoirs during the early summer months, a phenomenon of unbalanced nitrification is frequently observed, leading to accumulation of nitrite.

Unexpectedly, we found that nitrifiers, both *Nitrosomonas* sp. and *Nitrobacter* sp., are abundant in the anaerobic hypolimnion of these reservoirs, as well as in anaerobic stabilization ponds (Abeliovich 1987). In the laboratory, *Nitrosomonas europaea*, under strict anaerobic conditions, utilized nitrite as an electron acceptor, and pyruvate as energy source. Labeled pyruvate did not penetrate the cell membrane and was not incorporated into cell material, but its presence was essential for incorporation of $^{14}CO_2$ into cell material. Either pyruvate participates in a phosphoroclastic reaction at the outer surface of the cell membrane, or the extracted protons generate a membrane potential used for generating the energy needed by *Nitrosomonas* to survive anaerobic conditions (Abeliovich & Vonshak, 1992). Earlier, Poth (1986) described a *Nitrosomonas* producing N_2 from nitrite under microaerophilic conditions, and Yamanaka & Sakano (1980) demonstrated oxidation of hydroxylamine to nitrite, and its further disappearance by extracts of *N. europaea* under anaerobic conditions. The ecological significance of these observations is not yet clear.

Denitrification

The end product of nitrification is, as mentioned earlier, an environmental contaminant and a health hazard in its own right, and further treatment is required to eliminate nitrates through denitrification. This is a term usually used to describe respiration of microorganisms with nitrate or nitrite replacing oxygen as a terminal electron acceptor, with N_2O or N_2 accumulating as end products. Although it is most common under anaerobic conditions, microbial aerobic denitrification has been shown in recent years to be a wide spread phenomenon (Robertson & Kuenen 1990). With the currently increasing levels of aquifer pollution with nitrates, primarily due to leaching of fertilizers, values often reach levels which are prohibitive to human consumption.

Elimination of nitrates can be achieved by processes based on membrane technology which are, for practical applications, possible only when nitrate is the dominant ion, since there are no nitrate selective membranes. Therefore any process aimed at filtering nitrates will have to filter all soluble ions too, which makes it economically impractical. Microbial denitrification using various carbon sources

is, at present, the only selective process, with the added advantage that the end product is gaseous.

Microbial denitrification has been thoroughly reviewed in a book published recently (Revsbech & Sorensen 1990) and will not be discussed here. Attempts in Israel to apply enhanced *in situ* microbial denitrification to treat the contaminated coastal aquifer proved to be impractical, both in the laboratory and in field studies (Soares et al. 1989, 1991). A prerequisite for successful *in situ* denitrification is an environment which permits the free flow of water on one hand, and the free exchange of gases between air and water in a phreatic aquifer on the other. The coastal aquifer in Israel is made of sand, silt and clay which traps the gas liberated in the denitrification process as bubbles which clog and prevent the free flow of water. Therefore, the only practically feasible process in the case of a sandy aquifer should be built as an efficient above ground installation.

Summary

In the sequence of events leading from ammonia to N_2 during the process of biotransformation of inorganic nitrogen compounds, the weakest link, with respect to our knowledge and understanding of the organisms involved, is nitrification. In particular, this is true for the oxidation of ammonia to nitrite. The enzymes have not been thoroughly studied, and the enzymatic mechanisms have not been identified. Almost any biochemical and physiological aspect studied proved to be controversial, and major ecological questions still remain unanswered. Unless the structure and function of the various components of the process are worked out, progress in developing means for controlling nitrification will depend mainly on laborious trial and error and not on knowledgeable manipulation of this group of bacteria.

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