

Microbial Nitrate-Dependent Oxidation of Ferrous Iron in Activated Sludge

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A biological reduction of nitrate and nitrite was found to take place in activated sludge concomitantly with the oxidation of ferrous iron to ferric iron. This process was shown to be predominantly biological and present in different types of activated sludge treatment plants with variable rates. The highest activity was found in plants with biological nitrogen and phosphorus removal. The highest Fe(II)-dependent nitrate removal rate was found to be $0.31 \text{ mmol NO}_3^- (\text{g VSS})^{-1} \text{ h}^{-1}$, which corresponded to 68% of the maximum dissimilatory nitrate reduction rate in the presence of lactate. The Fe(II)-dependent nitrate removal rate was strongly pH-dependent with a maximal rate at pH 8 of almost four times the rate at pH 6. The main product of Fe(II)-dependent nitrate removal was most probably dinitrogen, as no accumulation of ammonia, nitrous oxide, or nitrite could be observed. The process may be of significance in the activated sludge treatment plant with regard to nitrate removal and with regard to the reoxidation of Fe(II) to Fe(III), which influences the chemical phosphorus removal and the flocculation properties of the sludge.

Introduction

Ferric iron is important as an oxidant for biological organic matter mineralization in various anoxic environments, such as sediments and soils. The produced ferrous iron can be reoxidized by a number of processes, where oxidation by oxygen is probably the most important and best described process. A significant chemical oxidation by oxygen may take place under neutral conditions, while mainly biological oxidation prevails under acidic conditions. In the absence of oxygen, other processes may oxidize ferrous iron. Fe(II) can be oxidized chemically by manganese(IV) oxide (1) and various nitrogen compounds such as nitrate, nitrite and nitrous oxide (also known as chemodenitrification). However, these abiological processes are believed to be of little significance, if any, in natural environments, except under extreme conditions such as elevated temperatures (2), in the presence of certain catalysts (3), or under acidic or alkaline conditions (4).

It is suggested that biological oxidation of Fe(II) by nitrate and nitrite occurs in natural environments. Straub et al. (5) showed that biological oxidation of ferrous iron with nitrate was carried out by enrichment cultures and pure cultures of some denitrifiers in the absence of oxygen as a light-independent, chemotrophic microbial process. However, the significance of this process in complex natural systems is still uncertain.

Recently, it was shown that nitrate was reduced in activated sludge from a wastewater treatment plant when ferrous iron was added (6). It was not clear whether the process was mainly chemical or biological, but since an activated sludge system provides perfect environmental conditions for the development of microbial communities with a capability to oxidize Fe(II) with nitrate or nitrite, it was hypothesized that it was mainly biological. A modern wastewater treatment plant with biological nitrogen and phosphorus removal provides periods where the microbial communities are simultaneously exposed to ferrous iron and nitrate or nitrite in the absence of oxygen (7, 8). Nitrate is produced by nitrification in aerobic periods, and ferrous iron is produced by microbial Fe(III) reduction (6, 9) under anaerobic conditions. Thus, in the subsequent denitrification period nitrate and Fe(II) are present together. Furthermore, iron is often present in large quantities because it is added to enhance chemical P removal (10).

This study was performed to investigate whether oxidation of ferrous iron by nitrate in activated sludge is mainly biological or chemical, whether it is a significant process in activated sludge, and to get more information about the stoichiometry and kinetics of the process.

Materials and Methods

Activated Sludge Sampling and Preparation. Most of the investigations were conducted on activated sludge from Aalborg West Wastewater Treatment Plant (WTP). It performs biological nitrogen and phosphorus removal with the alternating biondenitro process (7). In this type of treatment plant, the sludge is exposed to cycles with alternating phases of 2–4 h duration. One cycle consists of an oxic period, with nitrification as an important process, a denitrification period to remove nitrate, and an anaerobic period important for the biological P removal. In addition, the plant has simultaneous chemical P removal by addition of ferrous sulfate. Activated sludge was collected from the aerobic process tank. Sludge samples were stored at 4 °C for a maximum of 2 days. Before any experiments were performed, a preincubation step of the sludge took place to remove readily degradable organic substances that could cause a high background rate of organic matter-dependent denitrification. The sludge was incubated aerobically at 21 °C with atmospheric air flow and stirred for 2 h. Nitrification was inhibited to avoid high background levels of nitrate by addition of 5 mg/L *N*-allylthiourea (SERVA). Control experiments without addition of *N*-allylthiourea showed no effect on other processes. Aliquots of the sludge slurry (800 mL) were dispensed into 1-L Erlenmeyer flasks, and oxygen was removed under a constant stream of pure N₂ for 30 min at 21 °C and stirring at 150 rpm before any experiments were initiated. In addition to Aalborg West WTP, 10 other activated sludge treatment plants were investigated for potential Fe(II)-dependent nitrate reduction. Sludge samples were treated identically to sludge from Aalborg West WTP. Typical total suspended solids (SS) concentrations were 2–5 g/L with an organic content (volatile suspended solids, VSS) of 60–70% of SS. Details about these treatment plants are described in Table 1.

Oxidation of Fe(II) by Nitrate and Nitrite. NaNO₃, NaNO₂, or FeSO₄ were added to the anaerobic sludge from anaerobic stock solutions. FeSO₄ was added to a final concentration of ~2 mM unless otherwise stated. The oxidation of Fe(II) and the removal of NO₃⁻ and NO₂⁻ was followed by measuring changes in the concentration of Fe(II), NO₃⁻, and NO₂⁻ over time in preincubated sludge kept under a flow of pure N₂, at 21 °C, and with stirring of

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TABLE 1. Description of Different Activated Sludge Treatment Plants and Their Content of Iron (Fe_{tot})^a

	plant	process	sludge age*, days	addition of Fe	Fe_{tot} , mmol (g VSS) ⁻¹
1	Grindsted	C	5–10		0.24
2	Kongerslev	C	5–10		0.03
3	Ø. Hornum	C, CP	5–10	+	2.87
4	Slettestrand	C,N	10–15		0.33
5	Arentminde	C,N	10–15		0.38
6	Malmø BP	C,(N),(DN),BP	4–6		0.10
7	Aabybro	C,N,DN,CP	20–30	+	2.34
8	Fjerritslev	C,N,DN,CP	15–25	+	2.49
9	Marselisborg	C,N,DN,BP,CP	15–25	+	1.09
10	Aalborg West	C,N,DN,BP,CP	20–30	+	2.15
11	Malmø BNP	C,N,DN,BP	20–25		0.13

^a The investigations were performed within 2 weeks in October/November. ^b C, organic matter removal; N, nitrification; DN, denitrification; CP, chemical phosphorus removal; BP, biological phosphorus removal; (N) and (DN), only during summer and autumn (warm periods). ^c Sludge age: Average residence time of the sludge in the treatment plant.

150 rpm. Samples of the slurry were taken anaerobically and analyzed for NO_3^- , NO_2^- , and NH_4^+ after centrifugation (4500g for 5 min) and filtration (polycarbonate filter, Whatman 2122). $Fe(II)$ and total Fe (Fe_{tot}) were analyzed after extraction (see below). The controls included activated sludge that was either pasteurized (10 min at 80 °C) or cooled to 4 °C or sludge where sodium azide (0.1% w/v) was added. Controls without addition of either nitrate or $Fe(II)$ were run to measure background activity. Chemical redox processes were also tested in pure anaerobic H_2O with addition of either $Fe(II)$, NO_3^-/NO_2^- , or both. pH was adjusted by addition of NaOH or HCl directly to the activated sludge. The pH value was measured in parallel incubations and showed a small increase or decrease toward the original pH value. The change only accounted for ~0.2 pH units within the experimental period of 3 h. Change in pH due to addition of ferrous iron was not observed. Nitrate reduction and iron oxidation processes taking place in the extracellular matrix of the sludge were tested after extraction of extracellular polymers from the activated sludge in Aalborg West WTP. Activated sludge was extracted for 1 h by using the cation exchange method described by Frølund et al. (11), extracting approximately 10% of the organic matter.

$Fe(II)$ oxidation rates and NO_3^-/NO_2^- removal rates were determined from the initial slope the first 20–30 min after addition of the agent, thereby estimating the maximum potential rate. The rates were corrected for the background reduction/oxidation rate observed before addition of either $Fe(II)$ or NO_3^-/NO_2^- . The stoichiometry was calculated from these rates to avoid problems with iron-independent reduction of nitrate. N_2O -dependent $Fe(II)$ oxidation was performed in serum bottles, closed with chlorobutyl stoppers. N_2O was added to the headspace.

Potential $Fe(III)$ Reduction Rates and Denitrification Rates. In all activated sludge treatment plants investigated the potential $Fe(III)$ reduction rates were also measured. Lactate (2 mM) was used as an organic substrate, and the accumulation of $Fe(II)$ was measured over time (6). Moreover, in all plants, the potential denitrification rate was measured in the presence of lactate. Lactate was added to anoxic activated sludge, and the nitrate removal was recorded with time and the denitrification rate calculated. Sludge from two of the treatment plants (plant 7 and 10 in Table 1) were used in triplicate for determination of the nitrate removal rates with or without ferrous iron and lactate. Their relative standard deviation was below 5%.

Analytical Techniques. $Fe(II)$ was extracted anaerobically by ion exchange in 1 M anaerobic $CaCl_2$ (pH 7) overnight by a modification of the method described by Heron et al. (12). Aliquots of 2 mL of activated sludge were transferred anaerobically to serum bottles via syringes. The bottles were

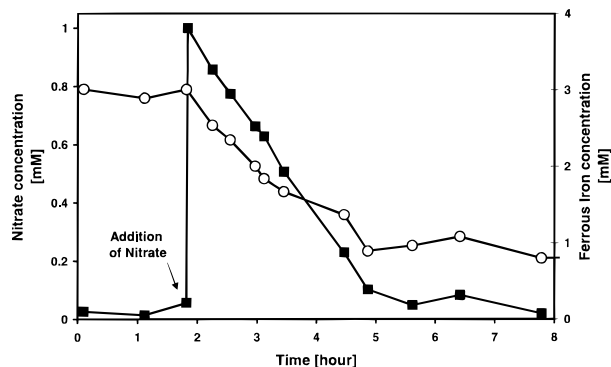


FIGURE 1. Changes in the concentration of $Fe(II)$ (○) and nitrate (■) in activated sludge after addition of nitrate to preaerated anaerobic activated sludge from Aalborg West WTP. SS and VSS of the sludge were 4.17 g/L and 2.63 g/L, respectively.

closed with chlorobutyl stoppers, evacuated, and refilled with pure nitrogen three times, and leaving a small excess pressure. Twenty milliliters (exact volume determined by weight) of an anaerobic $CaCl_2$ solution was injected into the bottles and shaken (150 rpm) on a rotary shaker overnight at 21 °C. The extractions were performed in triplicate with relative standard deviations below 5%. Control extractions without activated sludge, but with added ferrous iron and nitrate showed no oxidation or reduction in ferrous iron or nitrate. Total Fe (Fe_{tot}) was extracted in 0.5 N HCl according to Rasmussen and Nielsen (10). Fe_{tot} and $Fe(II)$ concentrations in the extracts were obtained spectrophotometrically by the Ferrozine method (10). Total suspended solids (SS) and volatile suspended solids (VSS) were determined according to APHA (13). NO_3^- , NO_2^- , and NH_4^+ concentrations were analyzed by an autoanalyzer (Technicon TRAACS 800 autoanalyzer). Detection limits for nitrate, nitrite, and ammonia were ~0.02 mM. Nitrous oxide was measured by sampling from the headspace of culture flasks using a gas chromatograph with electron capture detection. The Bunsen solubility coefficient (14) was used to calculate the total amount of N_2O accumulated in the sample bottles.

Results and Discussion

Biological Reduction of NO_3^- and NO_2^- by $Fe(II)$. When NO_3^- was added to anaerobic activated sludge from Aalborg West WTP with a background concentration of ~3 mM $Fe(II)$, an oxidation of $Fe(II)$ was immediately observed (Figure 1). Oxidation of $Fe(II)$ occurred until all nitrate was removed after 3 h of incubation. If nitrate was added in excess to the activated sludge, the ferrous iron was oxidized until approximately 1 mM remained, corresponding to around 30%

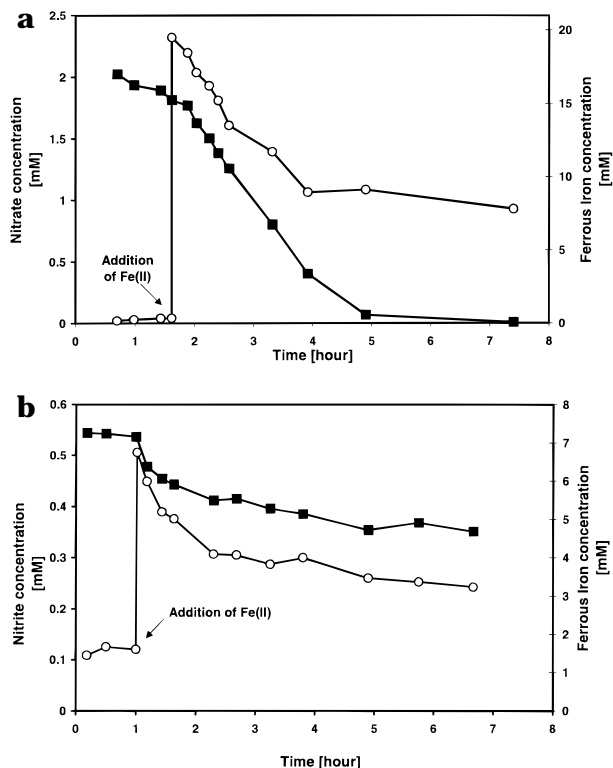


FIGURE 2. (a) Changes in the concentration of Fe(II) (○) and nitrate (■) in activated sludge after addition of nitrate and ferrous iron to preaerated anaerobic activated sludge. SS and VSS of the sludge were 3.32 g/L and 2.20 g/L, respectively. (b) Changes in the concentration of Fe(II) (○) and nitrite (■) in activated sludge after addition of nitrite and ferrous iron to preaerated anaerobic activated sludge. SS and VSS of the sludge were 4.75 g/L and 3.22 g/L, respectively.

of the initial concentration. The remaining concentration may have been too low, or the iron was not accessible for the microorganisms because it was bound in the floc matrix. However, most of it could easily be oxidized chemically by oxygen (6). Nitrite was also reduced by ferrous iron in activated sludge following a similar behavior as the nitrate reduction by ferrous iron (data not shown).

When Fe(II) was added to anoxic, denitrifying activated sludge, an increase in the nitrate removal rate (Figure 2a) or nitrite removal rate could be observed (Figure 2b). The figures show an increase in the removal rates immediately after addition of Fe(II). Most of the nitrogenous compounds were removed within the first 90 min, whereafter the removal rates approached the background removal rates observed before addition of Fe(II).

A number of controls were run to confirm that the observed oxidation was mainly biological. In pure anaerobic water, no oxidation of Fe(II) took place. After addition of nitrate or nitrite, no or only very little oxidation of Fe(II) was observed. Likewise, no or very little oxidation of Fe(II) was observed in pasteurized sludge (10 min at 80 °C), in activated sludge kept at 4 °C, or in sludge containing 0.1% (w/v) sodium azide (Figure 3). A control experiment with addition of 2 mM ferrous iron and 1 mM nitrite to sludge containing 0.1% sodium azide confirmed that no oxidation of ferrous iron or reduction of nitrite took place within 4 h. This result strongly indicates that nitrite produced by nitrate reduction was not abiotically reduced (data not shown). Furthermore, it was observed that the ability of the activated sludge to oxidize ferrous iron by nitrate or nitrite disappeared within a few days when the sludge was stored at 4 °C and brought back to normal temperature. It was also evaluated whether the

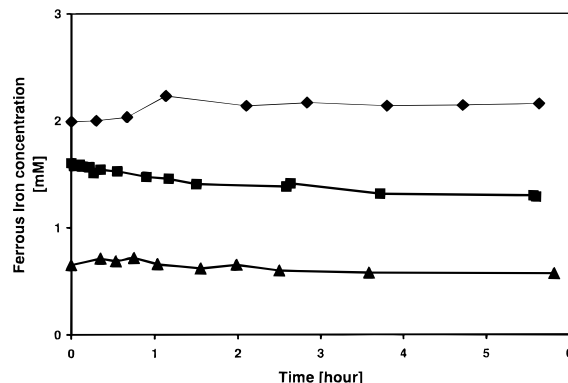


FIGURE 3. Changes in the content of ferrous iron after addition of nitrate (at time 0) to preaerated sludge containing sodium azide (◆), pasteurized sludge (▲), and sludge kept at 4 °C (■). SS and VSS of the sludge were 5.73 g/L and 3.86 g/L, respectively.

oxidation could take place in the extracellular matrix of the sludge flocs. Extracted exopolymers did not cause any significant removal of nitrate or oxidation of Fe(II) under anaerobic conditions, suggesting that the process was directly associated with the microbial cells.

The high rates of ferrous iron-dependent nitrate reduction observed confirms the suggestion by Straub et al. (5) that microbes may indeed be important for anoxic oxidation of ferrous iron with nitrate in a range of environmental systems. Activated sludge seems to be a very suitable model system to investigate a microbial community capable of performing this process. The way such treatment plants are operated provides excellent conditions for a range of different types of microorganisms, among which denitrifiers are an important group (8). Some of these denitrifiers, e.g., *Pseudomonas stutzeri*, belong to the microorganisms that are shown to be able to use ferrous iron as an alternative electron donor (5).

Product Formation. A number of experiments were conducted to identify the products formed during the nitrate reduction by oxidation of ferrous iron in the sludge samples (Figure 4). NH_4^+ was produced at a constant rate in the activated sludge before and after ferrous iron was added and thus independent of the reaction between nitrate/nitrite and ferrous iron. No accumulation of NO_2^- was observed as a result of the $\text{NO}_3^-/\text{Fe(II)}$ redox process (Figure 4a). Likewise, no nitrous oxide was produced in measurable amounts. It was also tested whether N_2O (1000 ppm) could oxidize ferrous iron in activated sludge, but no or very little oxidation was found. Furthermore, acetylene, which is an inhibitor of the reduction of N_2O to N_2 in the normal denitrification process (15), was added to the sludge in a final concentration of 10% (v/v) prior to addition of nitrate and ferrous iron. The addition of acetylene to sludge without addition of ferrous iron caused an accumulation of nitrous oxide, which could account for ~80% of the added nitrate. When ferrous iron was added to sludge containing nitrate and acetylene, nitrous oxide also accumulated, but in significantly lower levels.

When dissimilatory nitrate reduction took place in the sludge without addition of Fe(II), but with lactate as carbon source, a transient accumulation of some nitrite took place (Figure 4b). Nitrite accumulation is often observed in complex microbial systems, e.g., in activated sludge, when it is spiked with nitrate (16). Whether nitrite was an extracellular intermediate when ferrous iron was added is also not known, but if so, the reduction of nitrate must have been the rate-limiting step, as no nitrite accumulation could be detected. Addition of acetylene caused an accumulation of N_2O in agreement with other studies on dissimilatory nitrate reduction (15). The lower level of accumulation of NO_2^- and N_2O with the ferrous iron-dependent nitrate

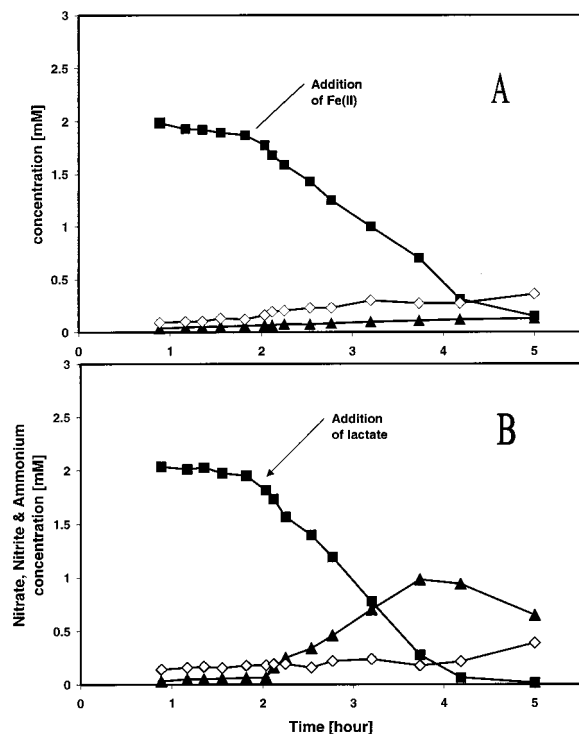
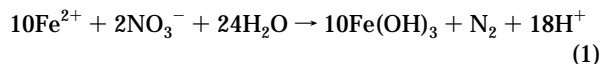


FIGURE 4. Comparison of the formation of nitrite (▲) and ammonia (◇) as a possible product from the reduction of nitrate (■) after addition of ferrous iron (A) and lactate (B) to pre-aerated anaerobic activated sludge. SS and VSS of the sludge were 2.56 g/L and 1.73 g/L, respectively.

reduction could indicate that the enzymatic pathways involved here are different from the organic matter-dependent nitrate removal.

The results strongly indicate that nitrate was reduced to dinitrogen in the sludge by the reaction with ferrous iron, as was also found by Straub et al. (5) in enrichment cultures and pure cultures. This was confirmed by the small accumulation of nitrite and nitrous oxide with or without acetylene present, as well as the lacking production of ammonia. If chemodenitrification had taken place, nitrous oxide would be expected to be the principal product at neutral pH as described by Buresh and Moraghan (3, 4).

Stoichiometry. The molar ratio between removed nitrate and oxidized ferrous iron was in the range of 0.20–0.44 mol of NO_3^- per mole of Fe(II) oxidized. This ratio was found in a number of similar experiments run over several months. This ratio was independent of the initial concentrations of NO_3^- and Fe(II) used (0–20 mM Fe(II) and 0–10 mM NO_3^-). Furthermore, in a survey of Fe(II)-dependent nitrate removal in 11 different treatment plants (see later), the ratio between the nitrate reduced and the ferrous iron oxidized was found to be between 0.24 and 0.36. The corresponding molar ratio for biological oxidation described by Straub et al. (5) was around 0.22, while Buresh and Moraghan (4) found very low values (0.08–0.13) for the chemodenitrification. The lowest observed molar ratio of reduced nitrate to oxidized ferrous iron was 0.20 in this study, which is in agreement with dinitrogen as the main product as suggested by Sørensen (17):



In most of the experiments there was a tendency to reach a higher ratio between NO_3^- and Fe(II). If the product of the nitrate reduction was nitrogen oxide, nitrous oxide, or

ammonia, the theoretical molar ratio would be 0.33, 0.25, and 0.13, respectively. Thus, in some of the experiments there seemed to be a higher removal of nitrate than could be expected from dinitrogen as the main product. Straub et al. (5) observed the same tendency and hypothesized that there might be a formation of complexes between oxidized nitrogen species and iron. However, another explanation could be that a stimulation of the background denitrification rate took place, which was assumed to remain at the same level as before addition of ferrous iron. A possible carbon source could be organic storage material, e.g., glycogen or PHB, which is common in activated sludge microorganisms (8). A third possibility is that some NO was produced in the experiment as has been observed by Sørensen (18). In the present study, NO was not analyzed for.

Oxidation Rates. The addition of ferrous iron to activated sludge containing nitrate or nitrite caused an increase in the nitrate or nitrite removal rate of up to 8 times the observed background removal rate. An example is given in Figure 2a, where the nitrate removal rate increased from 40 to 325 $\mu\text{mol NO}_3^- (\text{g VSS})^{-1} \text{h}^{-1}$. When nitrate was added to activated sludge containing Fe(II), the initial nitrate reduction rate was measured to 98 $\mu\text{mol NO}_3^- (\text{g VSS})^{-1} \text{h}^{-1}$ in the sludge (Figure 1). At the same time, the transformation of Fe(II) changed from a net Fe(III) reduction rate of 103 $\mu\text{mol Fe(III)} (\text{g VSS})^{-1} \text{h}^{-1}$ under anaerobic conditions to a net oxidation rate of Fe(II) of 1776 $\mu\text{mol Fe(II)} (\text{g VSS})^{-1} \text{h}^{-1}$ by addition of nitrate to Fe(II)-rich sludge. The nitrate removal rate induced by addition of Fe(II) corresponded to rates of up to 68% of the maximum dissimilatory nitrate reduction rate observed in the presence of lactate. This indicates that the population of microorganisms able to carry out this process was rather high, and/or that the microorganisms had a high specific activity.

The oxidation rate showed some dependence on the nitrate concentration, but it was not investigated in detail. The nitrate concentration used in the study is typical for wastewater treatment plants (0.1–1 mM), while the concentration of ferrous iron added (2 mM) was larger than normally observed in activated sludge, which is usually less than 1 mM (6, 9). It is important to realize that almost all ferrous iron is bound within the floc matrix (10), so the actual amount in the close vicinity of the bacteria is much larger than the 2 mM, which is an average concentration. The volume of activated sludge flocs after strong centrifugation is only around 1–2% of the entire volume, resulting in an actual Fe(II) amount corresponding to a concentration within the flocs of 100–300 mM. However, as the iron is bound in the EPS matrix, it is difficult to predict how this affects the kinetics of the reaction.

pH Dependency. A significant dependence on pH was observed in the ferrous iron-dependent nitrate reduction in the activated sludge. Figure 5 shows a pH optimum around 8 with a maximum rate of 132 $\mu\text{mol Fe(II)} (\text{g VSS})^{-1} \text{h}^{-1}$. This rate was ~4 times higher than the observed rate at pH 5. The strong pH dependency is typical for an enzymatic reaction with a maximum activity around pH 8 and with little activity at pH values above pH 9 and below pH 6. Whether this pH interval represents the activity of the enzymes involved or whether it corresponds to the activity of the microbial population is not known. In the activated sludge system examined, the pH in bulk water is always between 7 and 8.

Iron Redox Processes in Different Activated Sludge Treatment Plants. The presence of ferrous iron-dependent nitrate reduction was also investigated in a number of other activated sludge treatment plants. The treatment plants, shown in Table 1, represent the most common process designs and plant types. In all the treatment plants investigated, a microbial Fe(III) reduction could possibly take place under anaerobic conditions. The maximum potential Fe(III)

TABLE 2. Potential Fe(III) Reduction Rates after Addition of Lactate, Potential Nitrate Reduction Rate after Addition of Lactate, Fe(II) Dependent Nitrate Reduction Rates after Addition of Fe(II), and Background Nitrate Reduction Rates^a

plant	potential Fe(III) reduction (lactate), $\mu\text{mol Fe (g VSS)}^{-1} \text{h}^{-1}$	potential NO_3^- reduction (lactate) $\mu\text{mol NO}_3^- \text{(g VSS)}^{-1} \text{h}^{-1}$	background ^b NO_3^- reduction, $\mu\text{mol NO}_3^- \text{(g VSS)}^{-1} \text{h}^{-1}$	Fe(II)-dependent NO_3^- reduction, ^c $\mu\text{mol NO}_3^- \text{(g VSS)}^{-1} \text{h}^{-1}$	increase by Fe(II) addition, ^c %
1 Grindsted	23.8	223	81	103	28
2 Kongerslev	8.8	162	9	ND	ND
3 Ø. Hornum	25.6	229	96	106	11
4 Slettestrand	17.3	150	46	56	23
5 Arentminde	9.9	131	126	131	3
6 Malmø BP	22.8	530	150	310	107
7 Aabybro	56.8	271	101	146	45
8 Fjerritslev	42.9	160	54	69	28
9 Marselisborg	28.1	419	69	106	54
10 Aalborg West	56.8	373	68	130	91
11 Malmø BNP	17.0	290	60	120	100

^a The investigations were performed within 2 weeks in October/November. ^b Prior to addition of Fe(II). ^c ND: Not detectable.

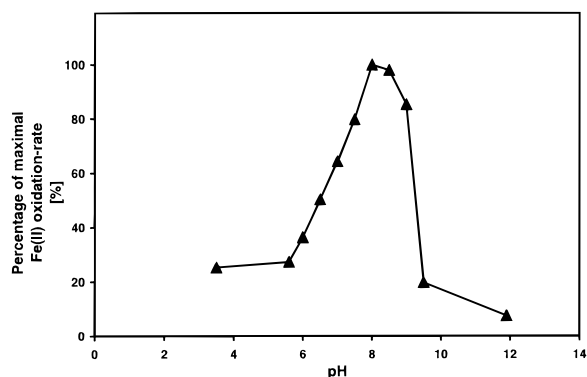


FIGURE 5. pH dependency of the Fe(II)-dependent nitrate removal rate in preaerated anaerobic sludge from Aalborg West WTP. SS and VSS of the sludge were 2.43 g/L and 1.63 g/L, respectively.

reduction rates in the presence of lactate ranged from 8.8 to 56.8 $\mu\text{mol Fe(III) (g VSS)}^{-1} \text{h}^{-1}$ (Table 2). These rates are in accordance with Fe(III) reduction rates found in other studies (6, 9).

In Table 2, the nitrate removal rates in the different treatment plants are shown. In all plants the potential nitrate removal rate (denitrification rate) was measured in the presence of lactate. The rates ranged from 0.1 to 0.5 mmol (g VSS)⁻¹ h⁻¹, which are typical values for denitrifying treatment plants (19). Similar rates were found by using acetate as electron donor (data not shown). Only five of the investigated treatment plants exhibited a significant Fe(II)-dependent nitrate reduction (plants 6, 7, 9, 10, and 11). The nitrate reduction rate in these plants was clearly enhanced by adding Fe(II) to the sludge, resulting in rates greater than the background nitrate removal rate. All these treatment plants but one (7) performed both N and biological P removal. Most of them also had a long sludge age, except Malmø Treatment Plant, which is a high-rate biological P removal plant with full N removal (nitrification and denitrification) only during the warm period of the year. This plant had the highest denitrification rate recorded, both in the presence of organic matter and in the presence of Fe(II). The Fe(II)-dependent rate at this plant was 310 $\mu\text{mol NO}_3^- \text{(g VSS)}^{-1} \text{h}^{-1}$. This was more than twice the rate found in any of the other plants.

None of the plants with a short sludge age and conventional organic matter removal had any significant Fe(II)-dependent nitrate reduction. This strongly indicates that alternating oxic, anoxic, and anaerobic conditions are necessary prerequisites to obtain microbial populations capable of Fe(II)-dependent nitrate reduction in the treat-

ment plants and that short sludge age (corresponding to a high organic loading) further promotes the activity of these microorganisms.

The ferrous iron-induced nitrate removal was measured several times throughout the year in Aalborg West Treatment Plant, and some temporal variations in the rates were found. The rates were highest during the late summer and early autumn and lowest throughout winter and spring as has also been observed for Fe(III) reduction in the same treatment plant (9). It is not known to what extent this also occurs in the other treatment plants included in the survey, and a possible temporal variation should be studied in greater detail.

Significance for the Wastewater Treatment Process. This study has confirmed the suggestions by Straub et al. (5) and Nielsen (6) that microbial Fe(II)-dependent nitrate reduction can be of significance in the environment. The process can be important particularly in activated sludge treatment plants with biological nitrogen and phosphorus removal. The reaction is of interest in relation to at least three processes in a typical treatment plant with nutrient removal. First of all, the process may be involved quantitatively in the denitrification process in addition to the organic matter-dependent nitrate reduction. In the investigation of treatment plants with an active Fe(II)-dependent nitrate removal, the rates ranged from 25 to 68% of the maximum nitrate removal rate that was observed in the plants when lactate was added (Table 2). This means that this process, if the conditions are optimal, can be almost as important for the denitrification process as the organic matter-dependent denitrification. The extent will depend on the concentration of Fe(II), nitrate, and organic matter, but the dependence on these factors is not well described so far and should be studied in greater detail.

Second, the chemical phosphorus precipitation in treatment plants relies on the presence of Fe(III), and for this reason, it is important that Fe(II) can be oxidized also under anoxic conditions, promoting an improved P removal. Finally, Fe(III) possesses better flocculation properties than Fe(II) (20). Therefore, Fe(II) added under anaerobic conditions (which is not unusual in many treatment plants for P removal) or produced by microbial Fe(III) reduction can be reoxidized in the presence of nitrate (e.g., in a denitrification tank), preventing deflocculation of the microbial flocs.

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