4. PROCESS CHEMISTRY AND BIOCHEMISTRY OF DENITRIFICATION

4.1 Introduction

The biological process of denitrification involves the reduction of nitrate nitrogen, NO_3^-, to a gaseous nitrogen species. The gaseous product is primarily nitrogen gas, N_2, but may also be nitrous oxide, N_2O, or nitric oxide, NO. Gaseous nitrogen is not readily available for biological growth, thus denitrification converts nitrogen to a harmless form which has no significant effect on the environment.

Some confusion has arisen in the terminology used in the literature. The process has been termed anaerobic denitrification. The principal biochemical pathways, however, are not anaerobic, but merely minor modifications of aerobic biochemical pathways. The term anoxic denitrification is therefore preferable, as it describes the environmental condition involving the absence of oxygen, without implying the nature of the biochemical pathways.

Denitrification is of interest because:

1. It is a major mechanism for loss of fertilizer nitrogen in agriculture, resulting in a decreased efficiency of the fertilizer.
2. It is of great potential application in the removal of nitrogen from high-nitrogen waste materials such as animal residues.
3. Many factors affect the accumulation of denitrification intermediates, such as N_2O, but only very few attempts have been made to develop a unifying explanation of the different intermediates.
4. Denitrification is the mechanism by which the global nitrogen cycle is balanced.
5. Most ground water resources of the world are facing a major nitrate contamination, which may result in infant methemoglobi .
6. It is a method for the removal of nitrogen from waste water.

The contribution of waste treatment systems to atmosheric N_2O is of some concern, because N_2O is involved in the stratospheric reactions, which result in the depletion of ozone, but little information is available. It is noteworthy, however, that fermentation, waste water acclimated to or supplemented with nitrate, released small quantities of N_2O during denitrification, whereas the waste water adapted to or

supplied with nitrite, produced none.

Nitrate contamination of ground water resources is becoming an ever increasing problem. Because of the adverse effects on health associated with nitrate in drinking water, and the concerns regarding diminishing water quality, the interest in nitrate removal technologies increases.

The drinking-water standard set by the U.S. Environmental Protection Agency (EPA), for nitrate is 10 mg/l as nitrate-nitrogen. The European Economic Community has a standard of 50 mg/l as nitrate (11,3 mg/l nitrate-nitrogen).

4.2 Types of Bacteria Accomplishing Denitrification

As distinct from nitrification, a relatively broad range of bacteria can accomplish denitrification. Genera of bacteria that are known to contain denitrifying bacteria include Pseudomonas, Micrococus, Archromobacter, Thiobacillus, and Bacillus (see Table 4.1). These bacteria are biochemically and taxonomically very diverse. Most are heterotrophs and some utilize one-carbon compounds, whereas others grow autotrophically on H_2 and CO_2, or on reduced sulphur compounds. Most of the mentioned bacteria possess the enzyme *reductase* necessary to reduce nitrate to gaseous nitrogen. But some lack the nitrate reductase enzyme and are termed nitrite dependent; and others lack N_2O reductase and thus yield N_2O as the terminal product. Still other organisms possess N_2O reductase but cannot produce N_2O from nitrate or nitrite. These different groups of bacteria also accomplish nitrate reduction by what is known as a process of nitrate dissimilation, whereby nitrate or nitrite replaces oxygen in the respiratory process of the organism under anoxic conditions. Because of the ability of these organisms to use either nitrate or oxygen as the terminal electron acceptor while oxidizing organic matter, these organisms are termed facultative heterotrophic bacteria.

Surprisingly, most of the organisms known to denitrify are not strict anaerobes, but rather facultative organisms, which under anoxic conditions use nitrate as a final electron acceptor. The sludge in combined nitrification and denitrification design processes is alternatively exposed to aerobic and anaerobic conditions, and because the denitrifying bacteria are facultative, the change of an oxic environment will provoke only minor adaptation problems.

Table 4.1 Genera of bacteria which are abundant in sewage and capable of performing denitrification.

Genera	*Abundant in sewage*	*Species within the genera are denitrifiers* $NO_3^- \rightarrow N_2$	*Only* $NO_3^- \rightarrow NO_2^-$
Achromobacter	Van Gils (1964)	Doelle (1969), Payne (1973), Smith *et al.* (1972)	
Aerobacter	Harris *et al.* (1927)		Doelle (1969)
Alcaligenes	Van Gils (1964), Harris *et al.* (1927)		
Bacillus		Smith *et al.* (1972)	
Flavobacterium	Van Gils (1964), Jasewicz and Porges (1956)		Payne (1973)
Micrococcus	Jasewicz and Porges (1956)	Payne (1973), Porra and Lascelles (1965)	
Proteus	Harris *et al.* (1927)		Payne (1973)
Pseudomonas	Jasewicz and Porges (1956)	Best and Payne (1965), Fewson and Nicholas (1961), Fry (1955), Payne (1973), Smith *et al.* (1972)	

Source: Henze Christensen and Harremoes (1977).

The fact that common sewage bacteria are denitrifiers makes it simple to create an appropriate environment for the denitrification process. All that is needed is the presence of nitrate, an electron donor (carbon source) and an anaerobic environment. A more specialized knowledge of species of bacteria is hardly necessary in most cases. Exceptions are where a special carbon source, such as methane, is used, as only very few bacteria can metabolize methane under anaerobic conditions.

Denitrifying bacteria can be identified according to the methods described in the Standard Methode (1985). Other are listed in Table 4.2.

4.3 Biochemical Pathways

Denitrification is a two-step process in which the first step is a conversion of nitrate into nitrite. The second step carries nitrite through two intermediates to nitrogen gas. This two-step process is normally termed "dissimilation".
Each step in the denitrification process is catalysed by a separate enzyme system.

Denitrifiers are also capable of an assimilation process whereby nitrate (through nitrite) is converted into ammonia. Ammonia is then used for the nitrogen requirements of the bacteria cells. The step or steps, from nitrite to hydroxylamine are not fully known.

Table 4.2 Methods for the identification of denitrifying bacteria.

Method	***Reference***
Chromatographic techniques	Payne (1973) Tood and Nuner (1973)
MPN-technique	Tood and Nuner (1973)
Measurements of the enzymatic activity plates	Lenhard (1969)

If ammonia is already present, for example in a nitrification plant, assimilation of nitrate need not occur to satisfy cell requirements.

The transfer of electrons from the carbon source (the electron donor) to nitrate or nitrite (the electron acceptor) to promote the conversion into nitrogen gas, will be discussed in detail in Section 4.4. It involves the "electron transport system" of the denitrifiers and consists of the release of energy from the carbon source for the use in the growth of the organism. This electron transport system is identical to that used for respiration by organisms oxidizing organic matter aerobically, except for one enzyme. Because of this very close relationship, many facultative bacteria can shift between using nitrate (nitrite) or oxygen rapidly and without difficulty.

Most investigators consider oxygen an inhibitor in the denitrification process. But some species have been reported to denitrify in systems with oxygen tension still as high as 0.2 atm. Table 4.3 show the metabolic processes in biological denitrification.

There is also evidence that nitrification and denitrification may occur simultaneously in soil or when applying special porous media, as for example clinoptilolite. Though not fully explained, these phenomena may occur in anaerobic micro-zones in otherwise aerobic systems (Masuda *et al.* 1987, 1990; Watanabe 1990; Halling-Sørensen and Hjuler 1992; 1993).

Many nitrate-reducing bacteria exhibit both dissimilatory and assimilatory behaviour. From an engineering point of view the ratio between dissimilated and assimilated nitrogen is of interest, as it is more desirable to produce nitrogen gas than to produce organic nitrogen bound in bacteria. Christensen and Harremoes (1977) and Painter (1970) indicate the yield coefficient for denitrifying bacteria $Y_{denit.}$ to be approximately 0,4 mg VSS per mg NO_3^- - N. If the nitrogen content in the organic matter is 10%, then 0,04 mg N is assimilated for every 1 mg NO_3^- - N converted into nitrogen gas.

An electron transport system for nitrate reduction is shown in Table 4.3, example 3. The steps from the electron donor to the cytochrome are always identical, while the final steps depend upon the final electron acceptor (nitrate, nitrite etc.).

Different species of bacteria may have slightly different electron transport systems, in particular in respect to quinone and cytochrome (Painter 1970).

For each of the steps in the dissimilatory nitrate reduction sequence a reductase enzyme has been isolated (Mudrack 1971).

Table 4.3 Metabolic processes in biological denitrification.

1: Dissimilatory nitrate reduction (denitrification).

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

2: Assimilatory nitrate reduction (synthesis).

$NO_3^- \rightarrow NO_2^- \rightarrow X \rightarrow NH_2OH \rightarrow$ Org. N

3: Possible electron transport system of the first step of denitrification.

e^- donor $\rightarrow$ NAD $\rightarrow$ FAD $\rightarrow$ Quinone $\rightarrow$ Cytochrome $\rightarrow$ Nitratereductase $\rightarrow NO_3^-$

4.4 Energy and Synthesis Relationship

The use of oxygen as the final electron acceptor is more energtically favored than the use of nitrate. By oxygen respiration the energy yield per mole of glucose is 686 kcal/mole and by nitrate dissimilation the energy yield per mole glucose is only 570 kcal/mole.

The greater free energy released for oxygen favors its use whenever it is available. Therefore, denitrification must be conducted in an anoxic environment to ensure that nitate, rather than oxygen, serves as the final electron acceptor.
Methanol, ethanol, acetic acid, have been most frequently used as the electron donor in experiments, rather than glucose.

Using methanol as an electron donor and neglecting synthesis, denitrification can be represented as a two-step process as shown in equations (4.1) and (4.2).
First step:

$$NO_3^- + 1/3\ CH_3OH \quad => \quad NO_2^- + 2/3\ H_2O \tag{4.1}$$

Second step:

$$NO_2^- + 0{,}5\ CH_3OH \quad => \quad 0{,}5\ N_2 + 0{,}5\ CO_2 + 0{,}5\ H_2O + OH^- \tag{4.2}$$

The overall transformation is obtained by addition of equations (4.1) and (4.2) yielding equation (4.3).

$$NO_3^- + 5/6\ CH_3OH \Rightarrow 0{,}5\ N_2 + 5/6\ CO_2 + 7/6\ H_2O + OH^- \quad (4.3)$$

Methanol serves as the electron donor in this equation and nitrate as the electron acceptor. This can be shown by splitting equation (4.3) into the following oxidation-reduction reactions.

Electron acceptor:

$$NO_3^- + 6\ H^+ + 5\ e^- \Rightarrow 0{,}5\ N_2 + 3\ H_2O \quad (4.4)$$

Electron donor:

$$5/6\ CH_3OH + 5/6\ H_2O \Rightarrow 5/6\ CO_2 + 5\ H^+ + 5\ e^- \quad (4.5)$$

It is clear from equations (4.4) and (4.5) that nitrate gains electrons and is reduced to nitrogen gas, which is the electron acceptor. The carbon source, in this example methanol, loses electrons and is oxidized to carbon dioxide, therefore it is the electron donor.

As mentioned in Section 3.4, these reactions take place in the context of the carbonic acid system. Equations (4.4) and (4.5) can be modified to reflect the fact that the hydroxide (OH^-) produced reacts with carbonic acid (carbon dioxide) to produce hydrogen carbonate alkalinity.

Nitrogen dissimilation and growth in denitrifcation reaction:

Nitrate to nitrite:

$$NO_3^- + 1/3\ CH_3OH \Rightarrow NO_2^- + 1/3\ H_2O + 1/3\ H_2CO_3 \quad (4.6)$$

Nitrite to nitrogen gas:

$$NO_2^- + 0{,}5\ CH_3OH + 0{,}5\ H_2CO_3 \Rightarrow 0{,}5\ N_2 + HCO_3^- + H_2O \quad (4.7)$$

Nitrate to nitrogen gas:

$$NO_3^- + 5/6\ CH_3OH + 1/6\ H_2CO_3 \Rightarrow 0{,}5\ N_2 + 4/3\ H_2O + HCO_3^- \quad (4.8)$$

Synthesis denitrification:

$$14\ CH_3OH + 3\ NO_3^- + 4\ H_2CO_3 \Rightarrow 3\ C_5H_7O_2N + 20\ H_2O + 3\ HCO_3^- \quad (4.9)$$

Combined dissimilatory-assimilatory equations for denitrification (after McCarty et al. 1969):

Overall nitrate removal:

$$NO_3^- + 1{,}08\ CH_3OH + 0{,}24\ H_2CO_3 \Rightarrow 0{,}056\ C_5H_7NO_2 + 0{,}47\ N_2 + 1{,}68\ H_2O + HCO_3^- \quad (4.10)$$

Overall nitrite removal:

$$NO_2^- + 0{,}53\ H_2CO_3 + 0{,}67\ CH_3OH \Rightarrow 0{,}04\ C_5H_7NO_2 + 1{,}23\ H_2O + 0{,}48\ N_2 + HCO_3^- \quad (4.11)$$

Overall deoxygenation:

$$O_2 + 0{,}93\ CH_3OH + 0{,}056\ NO_3^- \Rightarrow 0{,}056\ C_5H_7NO_2 + 1{,}04\ H_2O + 0{,}59\ H_2CO_3 + 0{,}056\ HCO_3^- \quad (4.12)$$

Equation (4.12) is shown since if any oxygen is present, it will be used preferentially before the denitrification.

The theoretical methanol requirement for nitrate reduction, neglecting synthesis is 1,9 mg methanol per mg nitrate-N (4.1). Including synthesis (equation 4.10) the requirement is increased to 2,47 mg.

Similarly, calculation of methanol requirements for nitrite reduction and deoxygenation allows a combined expression to be formulated for the methanol requirement.

$$Cm = 2{,}47 * NO_3^- - N + 1{,}53 * NO_2^- - N + 0{,}87 * DO \quad (4.13)$$

Where

Cm = required methanol concentration mg/l.

NO_3^- - N = nitrate-nitrogen concentration removed mg/l.

NO_2^- - N = nitrite-nitrogen concentration removed mg/l.

DO = dissolved oxygen removed mg/l.

The biomass X_B mg/l can be calculated similarly.

$$X_D = 0{,}53 * NO_3^- - N + 0{,}32 * NO_2^- - N + 0{,}19 * DO \quad (4.14)$$

For instance, for a NO_3^- value of 25 mg/l of nitrate-N, 0,5 mg/l nitrite-N and 3,0 mg/l dissolved oxygen, the methanol requirement can be calculated to be 64,1 mg/l from equation (4.13). The M/N ratio, which is the mg of methanol per mg of initial nitrate nitrogen concentration, is therefore 2,57 (64,1 / 25), which is only 4 percent greater then the requirement for nitrate alone.

Most experimental data is expressed in terms of the C/N ratio, which is the mg of carbon per mg of C per mg of initial nitrate-nitrogen concentration. The ratio includes the requirements for nitrite and oxygen, which are usually small relative to the nitrate requirement.

Values of the C/N ratio required for complete denitrification range from 1,5 to 5.

Table 4.4 show C/N ratio for different types of carbon sources used to perform denitrification. It has been suggested that column denitrification systems require a lower C/N ratio than suspended growth systems due to the higher concentration of biomass maintained in the column systems.

Higher biomass levels produce longer solids retention times and reduce organism yields due to increased endogenous metabolism. In turn this lower yield would result in less carbon required for synthesis and reduced C/N ratio.

In general, a C/N ratio of 2 to 3 will enable "complete denitrification" (95 % removal of nitrate) and this value may be used for design purposes when methanol is used as the carbon source for denitrification. Fig. 4.1 show the C/N ratio using methanol as carbon source as a function of the denitrification, in two different studies for submerged filters. The dotted line is the theoretical C/N ratio needed for total denitrification.

Table 4.4 C/N ratio for different types of carbon sources used to perform denitrification.

Organic matter	*C/N optimum*	*Unit*
as internal source	3-3,5	kg BOD/kg N
	4-5	kg COD/kg N
in sludge	1,5-2,5	kg BOD/kg N
	2,9-3,2	kg COD/kg N
Methanol	2,3-2,7	kg MeOH/kg N
	3,5-4,1	kg COD/kg N
Acetic acid	2,9-3,5	kg HAc/kg N
	3,1-3,7	kg COD/kg N

4.5 Alternative Electron Donors and the C/N Relationship

As shown in section 4.3, (equations 4.1 and 4.2), the denitrification process needs an electron donor to be accomplished.

A variety of compounds that can substitute for methanol as a carbon source have been evaluated experimentally and described in the literature. Table (4.5) shows the wide variety of carbon sources which have been used experimentally other than methanol and internal carbon.

The selection of an electron donor depends upon three factors which will be discussed in this section: availability of the electron donor, the reaction rate, and costs. The combination of a high reaction rate and moderate costs is achieved by the use of methanol.

Denitrification rates achieved with waste water organics, also called the internal carbon source, are approximately one third of those achieved when methanol is employed as the electron donor; this is because the availability of the electron donor is one of the most important factors controlling the activity of the denitrifiers. If the availability of the electron donor fluctuates, then the performance of the denitrification will also fluctuate, yielding a lower denitrification rate.

Denitrification reactors must, therefore, be proportionately larger using an internal carbon source than when methanol is used.

Volatile acids have also been used as a carbon source for denitrification. (Climenhage 1982). In studies of nitrate reduction in waste water generated in the manufacture

of nylon, is was found that a mixture of C_1 to C_5 volatile acids was very effective as a carbon source for denitrification.

It is also possible to use inorganic compounds as electron donors. Hydrogen and sodium sulphide have been used in these experiments (Kurt *et al.* 1987).

Some of the alternative carbon sources cause greater sludge production than others. About twice as much sludge is produced per mg of nitrogen reduced when saccharose is used, than when methanol is employed, because the yield coefficient of the bacteria using the first carbon source is greater.

On the other hand, acetone, acetate and ethanol produced similar quantities of sludge to that produced when methanol was employed.

Methanol has certain advantages over carbon sources in waste water. It is free of contaminants such as nitrogen, and can therefore be used directly in the process without taking special precautions that must be made for the use of a system with an internal carbon source. Using a external carbon source produces a consistent quality, while waste water sources may vary in strength and composition, either daily or seasonally, which complicates both process control and optimization. Use of waste water sources will require regular assays of the source to check its purity, and strength and its biological availability.

The disadvantage of using methanol is its cost, and this alone advocates the necessity of economic comparisons of alternate carbon sources.

Denitrification is considered to be a heterotrophic process, conducted by microorganisms that require a reduced organic substrate for energy and cell synthesis.

Heterotrophic denitrifying microorganisms can use a variety of organic carbon sources, while most of the published reseach regarding the denitrification of water, presumes the use of methanol, ethanol and acetic acid.

Figure 4.2 show the denitrification reaction rate as a function of temperature for different carbon sources. The more easily degradable the carbon source, such as methanol is, higher is the reaction rate. Heavily degradable endogenous carbon has a low reaction rate, especially at low temperature.

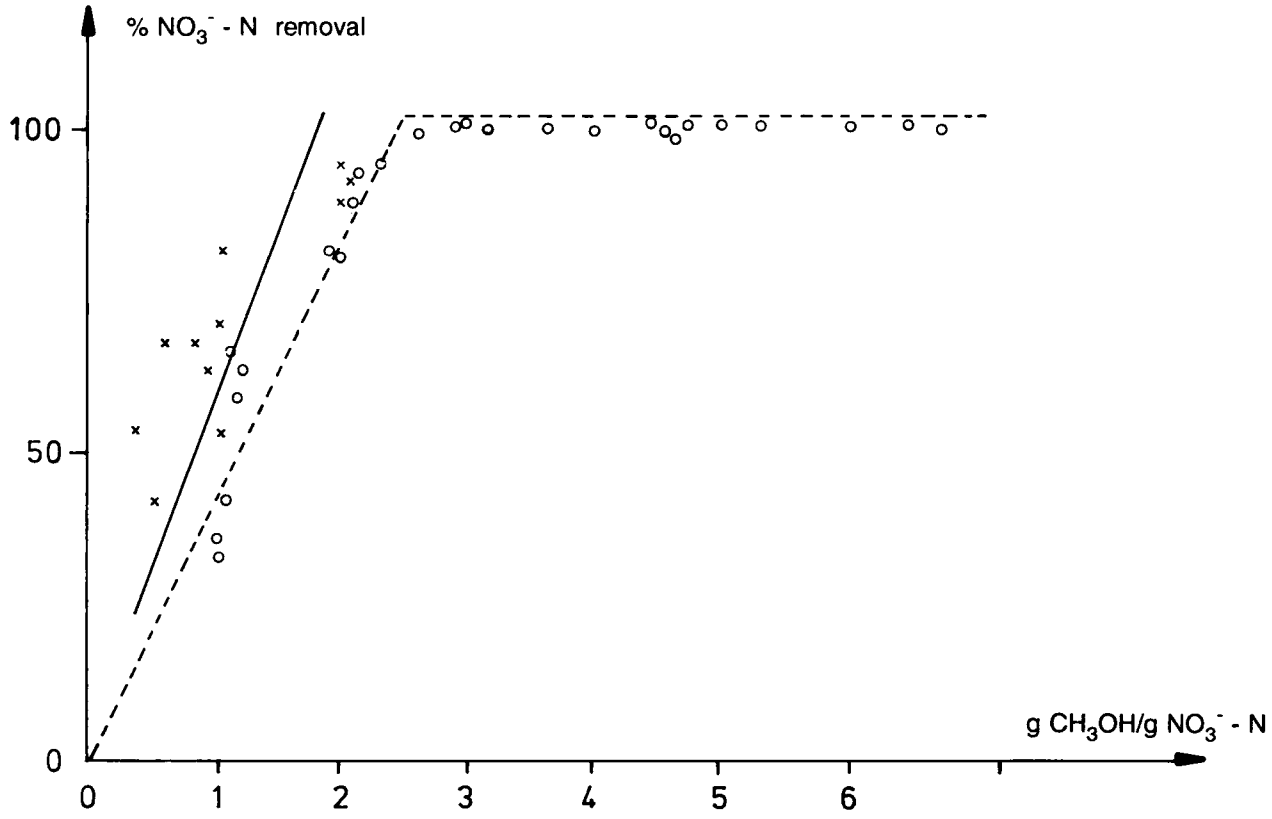

Fig. 4.1 C/N ratio using methanol as carbon source, in two different studies indicated as x and o, as a function of the denitrification efficiency, for a submerged filter. The dotted line is the theoretical amount
(Source: Henze and Harremoes 1978)

The stoichiometric relationships for these substrates have been formulated as follows:

Methanol (Sherrad 1988):

$$5\ CH_3OH + 6\ NO_3^- \Rightarrow 3\ N_2 + 5\ CO_2 + 7\ H_2O + 6\ OH^- \quad (4.15)$$

Ethanol (similar to equation 4.3)(Richard *et al.* 1980):

$$5\ C_2H_5OH + 12\ NO_3^- \Rightarrow 6\ N_2 + 10\ CO_2 + 9\ H_2O + 12\ OH^- \quad (4.16)$$

Acetic acid (Frick and Ricard 1985):

$$5\ CH_3COOH + 8\ NO_3^- \Rightarrow 4\ N_2 + 10\ CO_2 + 6\ H_2O + 8\ OH^- \quad (4.17)$$

Glycol (Monteith *et al.* 1980):

$$0{,}5\ (CH_2OH)_2 + NO_3^- \Rightarrow 0{,}5\ N_2 + CO_2 + H_2O + OH^- \quad (4.18)$$

Formaldehyde (Monteith *et al.* 1980):

$$1{,}25\ HCHO + NO_3^- \Rightarrow 0{,}5\ N_2 + 1{,}25\ CO_2 + 0{,}75\ H_2O + OH^- \quad (4.19)$$

Isopropanol (Montheith *et al.* 1980):

$$0{,}278\ C_3H_7OH + NO_{3-} \Rightarrow 0{,}5\ N_2 + 0{,}833\ CO_2 + 0{,}611\ H_2O + OH^- \quad (4.20)$$

Fusel oil (as amyl alcohol) (Montheith *et al.* 1980):

$$0{,}167\ C_5H_{11}OH + NO_3^- \Rightarrow 0{,}5\ N_2 + 0{,}833\ CO_2 + 0{,}5\ H_2O + OH^- \quad (4.21)$$

Dextrose (Montheith *et al.* 1980):

$$0{,}208\ C_6H_{12}O_6 + NO_3^- \Rightarrow 0{,}5\ N_2 + 1{,}25\ CO_2 + 0{,}75\ H_2O + OH^- \quad (4.22)$$

Gaseous organic substrates, such as methane and carbon monoxide, can also be used as substrates in denitrification. Among gaseous substrates, methane is one of the most studied; but some contradictions remain in the literature regarding methane metabolism. There is evidence that methane can be used as a terminal electron acceptor by some denitrifiers (Davies 1973).

Other investigators have suggested that methane oxidation requires aerobic or microaerophilic conditions, and that subsequent denitrification may be the result of a symbiotic relationship between two groups of organisms with different trophic requirements (Yull-Rhee *et al.* 1978).

It is likely that both phenomena occur, indicating two possible mechanisms for methane utilization during denitrification. Fewer studies have been published involving carbon monoxide, but there is evidence that it can be used as a substrate for denitrification (Park and Hegeman 1984).

Stoichiometric relationships for methane and carbon monoxide utilization have been proposed.

Methane (Barrenstein *et al.* 1986):

$$5\ CH_4 + 8\ NO_3^- + 8\ H^+ \Rightarrow 5\ CO_2 + 4\ N_2 + 14\ H_2O \quad (4.23)$$

Carbon monoxide:

$$2\ NO_3^- + 5\ CO + H_2O \Rightarrow N_2 + 2\ OH^- + 5\ CO_2 \quad (4.24)$$

Denitrification can also be accomplished by autotrophic bacteria, which can use hydrogen or various reduced-sulphur compounds as energy sources. Under autotrophic growth conditions, no organic carbon sources are required, rather carbon dioxide or bicarbonate is used as a carbon source for cell synthesis.

Paracocus denitrificans and *Thiobacillus denitrificans* can denitrify using hydrogen and reduced-sulphur compounds, respectively. Both of these bacilli can also grow heterotrophically, if an organic carbon source is present.

The following stoichiometric relationships for hydrogen and sulphur have been reported:

Hydrogen (Kurt *et al.* 1987):

$$2\ NO_3^- + 5\ H_2 \Rightarrow N_2 + 4\ H_2O + 2\ OH^- \quad (4.25)$$

Thiosulphate (Claus and Kutzner 1985):

$$5\ S_2O_3^{2-} + 8\ NO_3^- + H_2O \Rightarrow 4\ N_2 + 10\ SO_4^{2-} + 2\ H^+ \quad (4.26)$$

Sulphide (Barrenstein *et al.* 1986):

$$S^{2-} + 8\ NO_3^- + 8\ H^+ \Rightarrow 5\ SO_4^{2-} + 4\ N_2 + 4\ H_2O \quad (4.27)$$

The C/N relationship decribes the quantity of organic matter, which is needed per unit of nitrate-nitrogen that is converted to nitrogen gas by denitrification.

Organic matter of many kinds (as shown in Table 4.5) can be used for the following three purposes in a denitrification plant.

1) Reduction of nitrate or nitrite into nitrogen gas.
2) Sludge production, i.e. biomass production.
3) Respiration with oxygen.

Knowing the values of the three parameters described, it is possible to quantify the C/N relationship for a denitrification plant.

If the C/N ratio is smaller than is stoichiometrically needed, the denitrification process will not proceed or be applied with reduced capacity. If there is less nitrate or nitrite it will be converted into nitrogen gas.

Monteith *et al.* (1980) conducted an experiment in 30 industrial waste water streams. Twenty-seven of the 30 industrial waste streams were evaluated as external sources of carbon, added to domestic waste water. Fifty per cent of the waste water tested supplied a sufficient content of carbon for a constant denitrification of domestic waste water and exhibited denitrification rates equal to or greater than those observed using methanol. The C/N ratio found in the described experiments with external sources of carbon were between 0,7 to 2,6 kg FOC/kg NO_T-N removed. If methanol were used at carbon source an average of C/N ratio was found to be 1,17 kg FOC/kg NO_T-N removed. FOC is the amount of fully oxidisable carbon. NO_T - N er the total amount of nitrate and nitrite.

Table 4.5 Carbon sources other than methanol and internal carbon source in denitrifying experiments.

Compound	*Reference*
Acetic acid	Ide *et al.* (1972) Kiff (1972) McCarty (1969)
Acetone	McCarty (1969)
Alanine	Ide *et al.* (1972)
Bakery sludge	Adams *et al.* (1970)
Bouillon/Casein	Clayfied (1974) Edholm *et al.*(1970) Ericsson *et al.*(1966)
Brewery waste	Wilson and Newton (1973)
Chemical industry waste	Englehart and Haltrich (1968) Haltrich and Jager (1963, 1970)
Cherry juice	Adams *et al.*(1970)
Citrate	Ide *et al* (1972)
Corn starch	Adams *et al.*(1970) Ide *et al.* (1972)
Ethanol	Bringmann *et al.* (1959) Finsen and Sampson (1959) McCarty (1969) McCarty *et al.* (1969)
Fish meal	Ludzack and Ettinger (1962)
Gelatine	Ludzack and Ettinger (1962)
Glucose	Balakrishnan (1968) Balakrishnan and Eckenfelder (1969) Barth and Ettiger (1967) Christenson *et al.* (1956) Clayfied (1974)

Table 4.5 (continued)

	Ide *et al.* (1972) McCarty (1969) Schroeder and Busch (1967, 1968) Wuhrmann (1960)
Lactate	Ide *et al.* (1972) Toit and Davis (1973)
Margarine	Bringmann *et al.* (1959)
Methane	Christensen (1972) Harremoes and Christensen (1971) Parker *et al.* (1975) Pretorius (1972)
Milk solids	Aguirre and Gloyna (1967) Hermann (1962) Parker *et al.* (1975) Pretorius (1972)
Molasses	Finsen and Sampson (1959)
Nitro-cellulose waste	Mudrack (1971)
Peptone	Clayfied (1974) Ide *et al.* (1972)
Saccharose	Das *et al.* (1966) Finsen and Sampson (1959) Klotter (1969) McCarty (1969)
Sodium citrate	Dawson and Murphy (1972)
Sugary syrup	Adams *et al.* (1970)

Source: Henze Christensen and Harremoes (1977)

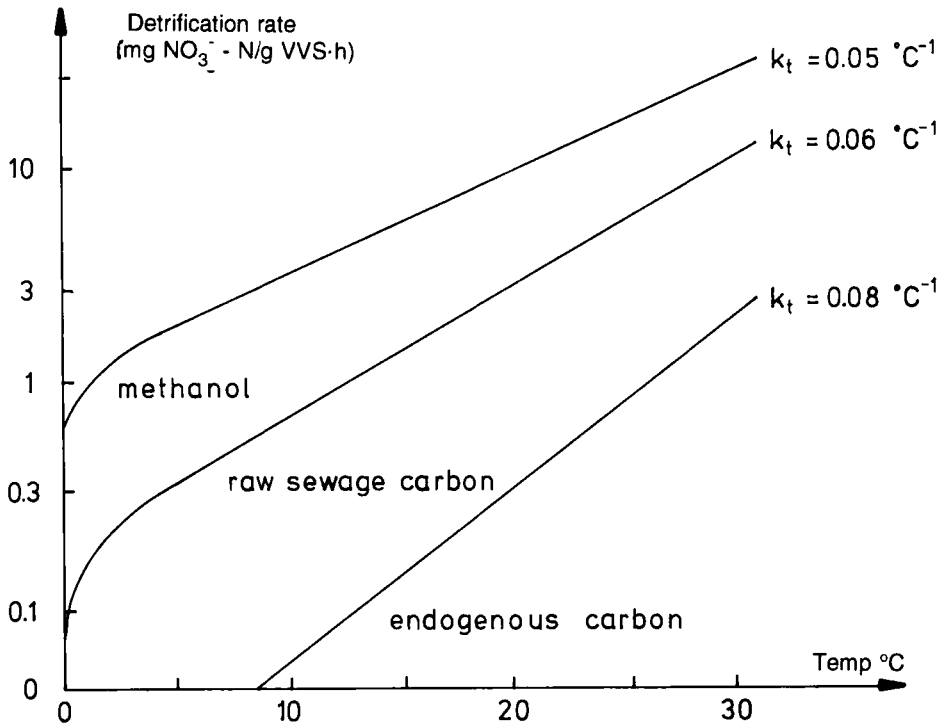

Figure 4.2 The denitrification reaction rate as a function of temperature for different carbon sources. The more easily degradable the carbon source, such as methanol the greater is the reaction rate. Heavily degradable endogenous carbon has a low reaction rate, especially at low temperature. (Source: Henze and Harremoes 1978)

4.6 Kinetic Expression for the Denitrification Process

Environmental factors also have a significant effect on the kinetic rates of denitrifier growth and nitrate removal. Temperature, pH, carbon concentration and substrate concentration are considered below. A combined kinetic expression considering factors that affect denitrification is proposed.

As in the case of nitrification the Monod Kinetic, equation (4.28), has also been proposed to explain the rate of conversion of nitrate to nitrogen gas, by several investigators, for example Henze and Harremoes (1972) and Moore and Schroeder (1970).

$$\mu_D = \mu_{max,D} * \frac{S_{denit}}{K_D + S_{denit}} \qquad (4.28)$$

where:

μ_D = growth rate for the denitrifier, day^{-1}.

$\mu_{max,D}$ = maximum growth rate for the denitrifier, day^{-1}.

S_{denit} = concentration of substrate to be denitrified (nitrate nitrogen) in mg/l.

K_D = saturation constant mg/l nitrate nitrogen.

Even though the Monod Kinetics is used by several investigators to explain the denitrification, the suspended denitrification process with methanol as carbon source is often described in the literature as zero order with respect to nitrate and methanol. The equation used in following this approach is presented as (4.36).

Denitrification filters appear to conform very well to the laws of biofilm kinetics. Because of the low saturation constant, K_s < 1 mg NO_3^--N / liter, the intrinsic reaction in the biofilm is zero order. This becomes a half-order reaction in thick biofilms owing to diffusional resistance in the biofilm. Where the substrate concentration in the bulk liquid is high enough, the biofilm is penetrated fully, and the overall process becomes zero-order (Harremoes 1982).

4.7 Relationship Between Growth and Removal Rate

Using the Monod Kinetic approach, denitrification rates can be related to the organism growth rates by the following relationship:

$$\frac{dS_{denit}}{dt} = -\frac{\mu_{max,D}}{Y_D} * \frac{S_{denit}}{K_D + S_{denit}} * X_D \qquad (4.29)$$

where X_D = biomass of the nitrifier bacteria and Y_D the yield coefficient.

4.8 Kinetic Constants in the Denitrification Process

The value of the saturation constant K_D is very low. Davies (1973), found the K_D value for suspended growth systems to be 0,08 mg/l nitrate nitrogen without solids recycling. For attached growth systems the value of K_D was found to be 0,06 mg/l nitrate nitrogen at 25 °C. Using these small K_D values in equation (4.29), that is S_{denit} is above 1-2 mg nitrate nitrogen, the denitrification will approach a zero order rate.

Several investigators (Christensen and Harremoes 1972; Stensel *et al.* 1973; Murphy and Dawson 1972; More and Schroeder 1970) have all reported zero order rates for the denitrification process, when the substrate concentration is above 1-2 mg/l N. Table 4.6 show kinetic constans for the denitrification process. The low value of the saturation constant, K_D, indicates that the denitrification process can be operated at near maximum unit removal rates and still give an acceptable nitrogen removal.

Table 4.6 Examples of kinetic constants for the denitrification process, using methanol as carbon source.

	10°C	20°C
K_s mg/l	12.6	9.1
K_d d^{-1}	0.05	0.04
Y_D g VSS / g COD	0.17	0.18

Source: Stensel and Bernard (1992)

4.9 The Influence of Oxygen on the Denitrification Rate

Investigators have reported various results for the influence of oxygen on the biochemistry of the denitrification process. Dissimilatory nitrate reduction (denitrification) is inhibited by oxygen, whereas assimilatory nitrate reduction is unaffected.

Payne (1973) explains that oxygen either represses the formation of the enzyme nitrate reductase or acts just as an electron acceptor, thereby preventing the reduction of nitrate.

Beneficial effects of oxygen in the denitrification process have been observed by Ide *et al.* (1972). The activity of denitrifying organisms seems to be enhanced after exposure to oxygen. This effect could be explained by the presence of haem in the electron transport system, as some organisms need oxygen in order to synthesize haem (Porra and Lascelles 1965; Tanaiguchi 1961).

The exact control mechanism exerted by oxygen on denitrifying enzyme synthesis, has not been clearly demonstrated yet, and may very well vary among species of denitrifiers.

When using attached cultures, it is especially important to distinguish between oxygen tension within the micro-environment around the bacteria, and oxygen tension within the macro environment.

It appears that 1-2 mg O_2/l does not influence denitrification in filters; but in suspended cultures the oxygen concentration should be below 0,5 mg O_2/l. Table 4.7 show the oxygen concentration in various denitrifying experiments.

4.10 The Influence of Temperature on the Denitrification Rate

Denitrification can be performed in the temperature range 5 °C - 35 °C. Many of the denitrifying species are adaptive to temperature changes.
It is, therefore, important to realize that there is a difference between long-term and short-term temperature influences on the denitrification process.

The growth rate of the organism and removal rate of nitrate are both affected by temperature. To show the effect of temperature on growth and denitrification rates, the results at 20 °C from the literature are summarized in Fig.4.3. Denitrification proceeds at a reduced rate, at temperatures as low as 5 °C. Above 20 °C, the data indicates that the denitrification rates are constant. Murphy *et al.* (1973) showed that attached growth systems are less affected by low temperatures than are suspended growth systems. It is important to distinguish between two types of temperature

Table 4.7 Oxygen concentration in denitrification experiments, and literature concerned with the technical importance of oxygen concentration.

Oxygen concentration in experiments (mg/l)	***Reference***
< 0,5	Ludzack and Ettinger (1962)
0,5 / 10,0	Ruffer (1964)
< 0,5	Pasveer (1965)
0,5	Schuster (1970)
0,2 - 5,0	Dholakia *et al.* (1970)
0,0 - 0,2	Carlson (1971)
0,0 - 1,5	Matschè (1971)
1,5 - 1,8	Smith *et al.* (1972)
0,0 - 2,5	Jones (1972)
0,0 - 2,0	Haltrich (1972)
0,15 - 0,72	Toit and Davies (1973)
< 0,2	Christensen (1973)
0 - 0,3	Drews and Greef (1973)
< 1,5	Parker *et al.* (1975)

Source: Henze Christensen and Harremoes (1977).

responses during denitrification, as described in Section 3.8 on the influence of temperature on nitrification.

The first type of response is an immediate (rapid) temperature response, which is much smaller than the long-term (slow) temperature response. The second type is the most interesting one; the former is the one often encountered in laboratory experiments. The long-term temperature response is a mixture of an immediate temperature response and adaptation of the microorganisms (Henze and Harremoes 1978).

Very little is known about the relationship between long-term and short-term temperature dependencies.

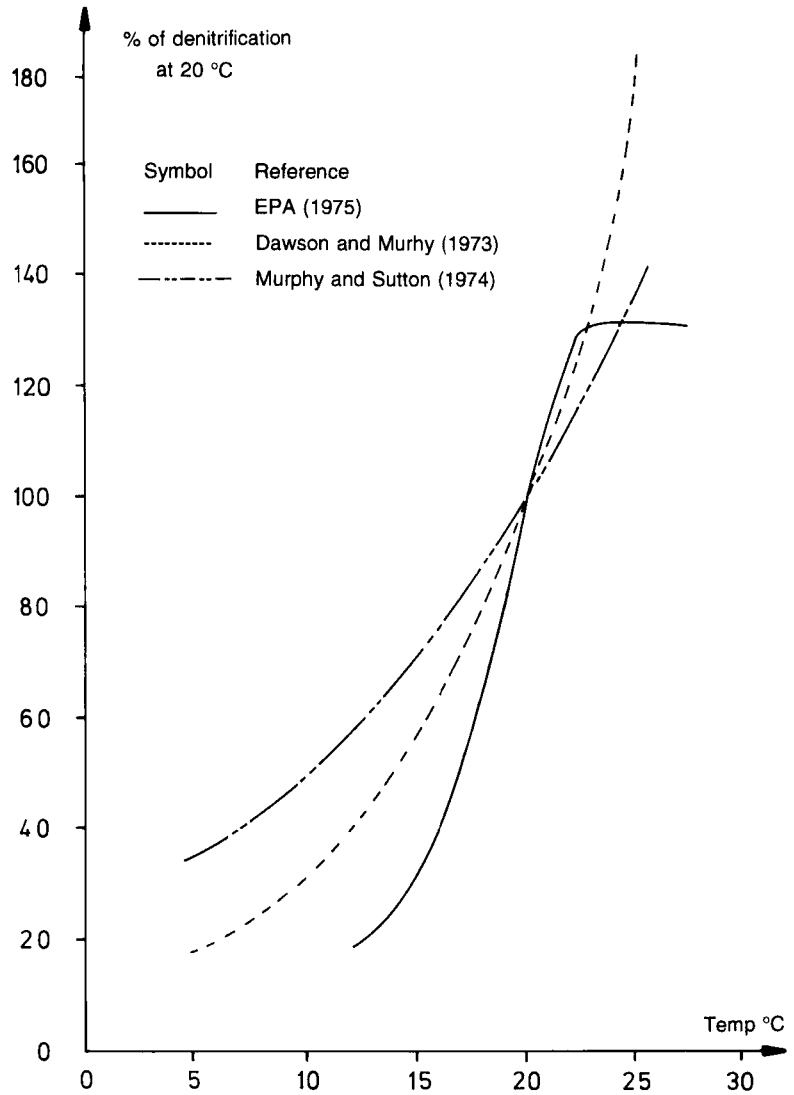

Figure 4.3 Temperature dependence of the denitrification process.

Mathematically, the dependence on temperature can be described by the following exponential expression:

$$\mu_{max,D_t} = \mu_{max,D_{20°}} \cdot \gamma^{t-20°} \tag{4.30}$$

where: Y is the temperature coefficient in Table 4.8.

The expression is valid only within the range from 5 ° C to about 35 °C. As the temperature in most cases changes slowly, long-term temperature dependencies are the most important for practical purposes.

In table 4.8, the long-term temperature constants k_t and θ for various denitrification processes are listed.

The following temperature expression is proposed by Hultmann (1971):

$$\mu_{max,t}=\mu_{max,20°C}*10^{k(t-20)} \tag{4.31}$$

According to Table 4.8 the literature shows that the temperature dependency for attached growth is smaller than that for suspended growth.

4.11 The Influence of Carbon Concentration on the Denitrification Rate

The effect of carbon concentration on the rate of denitrification has been explained with a Monod type of expression by, Stensel *et al.* (1973). Using methanol as the carbon source, the following expression was employed:

$$\mu_D=\mu_{max,D}*\frac{M}{K_M+M} \tag{4.32}$$

where:

M = methanol concentration, mg/l

K_M = saturation constant for methanol, mg/l.

The kinetic value of K_M is normally very low, normally in the order of 0,1 mg/l methanol.

Table 4.8 The influence of temperature on denitrification rate.

Process	*Carbon Source*	k_t -1 ^oC	*Y*	*Temp. range ^oC*	*Reference*
Suspended separate culture	Methanol	0,05	1,12	10-25	Henze and Harremoes (1977) Mulbager (1971)
Suspended combined culture	Raw sewage	0,06	1,15	5-20	Henze *et at.* (1977)
Suspended combined culture	Endogenous	0,08	1,20	15-25	Bernard (1975)
Attached separate culture	Methanol	0,02	1,05	5-20	Harremoes and Rimer (1977)
Attached culture	-	0,03	1,07	18-29	Mechala *et al.* (1970)

Table 4.8 (continued)

Process	*Carbon Source*	k_t -1 ^oC	*Y*	*Temp. range ^oC*	*Reference*
Suspended	-	0,06	1,15	11-21	Hünerberg and Sarfert (1967)
Suspended	-	0,05	1,12	10-20	Mulbager (1971)
Suspended	-	0,05	1,12	10-20	Stensel (1971)
Suspended	-	0,05	1,12	5-27	Dawson and Murphy (1972)
Suspended	-	0,05	1,12	10-40	Ide *et al.* (1972)
Suspended	-	0,03	1,07	6-25	Murphy and Sutton (1974)
Attached	-	0,03	1,07	6-25	Murphy and Sutton (1974)
Suspended	-	0,04	1,10	5-25	Sutton *et al.* (1975)
Suspended	-	0,07	1,17	15-24	Parker *et al.* (1975)

4.12 The Influence of pH on the Denitrification Rate

Denitrification only partially offsets the alkalinity loss caused by nitrification, as the alkalinity gain per mg of nitrogen is only one-half of the loss caused by nitrification. This is because the alkalinity gain per mg of nitrogen is only one-half the loss caused by nitrification.

A value for alkalinity production suitable for engineering calculations would be 3,0 mg alkalinity as $CaCO_3$ produced per mg nitrogen reduced.

In the design of systems where alternating nitrification and denitrification are used, a sudden high load of ammonia in the waste water can cause a self-destruction of the system, because of the high H^+ concentration developed during nitrification (Fig. 4.4) The denitrification will not occur because of the decreased pH, as the denitrifying organisms can not denitrify under a low pH condition.

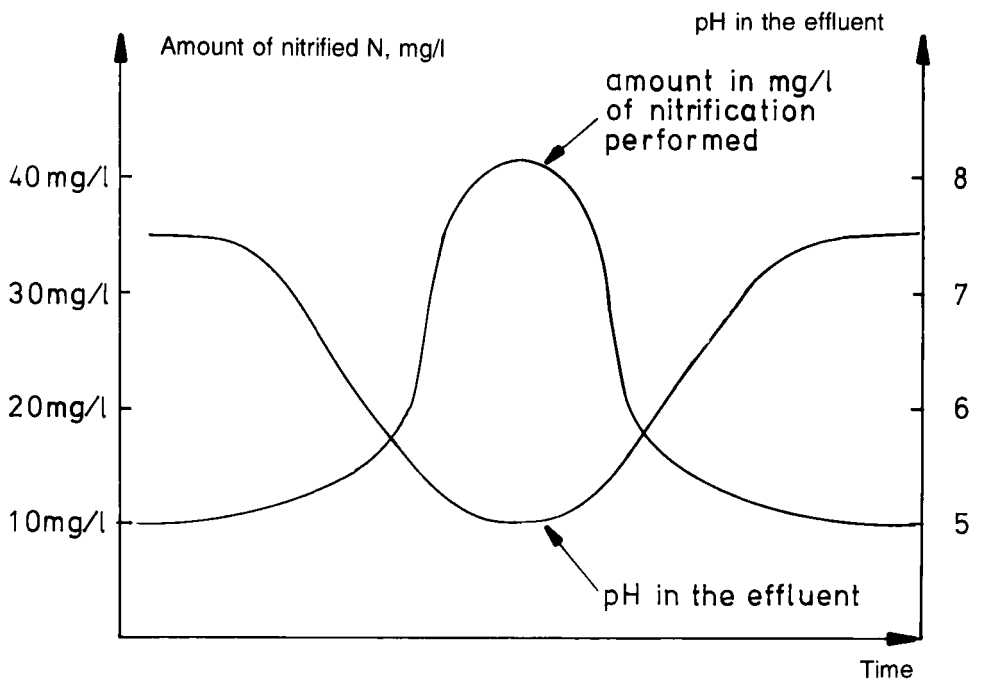

Figure 4.4 Self-destruction of a system applying alternated nitrification and denitrification due to a high H^+ production during nitrification.

Table 4.9 presents observations from the literature of the effect of pH on denitrification rates. It would appear that for most systems the denitrification rate is depressed below pH 6,0 and above pH 8,0. Different studies indicate different pH values as the optima for denitrification, but most studies show the highest rates of denitrification occur within the range of pH 7.0 to 7.5.

All results are presumably long-term pH dependence studies, but this is impossible to determine from the information available.

The influence of pH on denitrification is also dependent upon the duration of the effect. The short-term effect of a pH change is the most interesting, because a pH change generally does not vary over a long period.

In Section 4.3 it is shown that denitrification produces alkalinity, which will result in an increase in the pH value. The magnitude of this increase depends upon the buffering effect of the sewage, because nitrification, on the other hand, produces acidity.

In a combined nitrification-denitrification process, the pH of the two processes should thus balance each other out, the result being a constant pH. (Barth *et al.* 1968; Halling-Sørensen and Hjuler 1992).

Timmermann and Van Hauten (1983) determined the growth rate μ as a function of pH in batch reactors at 25 °C. The biomass of the bacteria was measured as a MLVSS- concentration. Figure 4.5 shows that a maximum growth rate was found at pH 8,5.

According to Hartmann and Laubenberger (1968), a deviation of the pH from the optimum pH reduces the bacterial activity according to the mechanism of non-competitive inhibition (see Section 3.13).

Table 4.9 pH variation in denitrification experiments, and pH studies.

pH-interval	***Reference***
7,0 - 9,0	Hermann (1962)
7,2 - 7,5	Johnson and Schroepfer (1964)
6,5 - 7,5	Meiring and Stander (1964)
7,9 - 8,1	McCarty (1969)
7,2 - 8,0	Barth and Ettinger (1968)

(Table 4.9 continued)

6,5 - 7,5	Moore (1969)
6,0 - 9,0	Renner (1970)
7,5 - 8,1	Hamm (1970)
6,0 - 10,0	Edholm *et al.* (1970)
7,4 - 9,1	Stensel (1971)
6,0 - 8,0	Mulbager (1972)
5,5 - 8,5	Kiff (1972)
6,0 - 10,0	Ide *et al.* (1972)
5,0 - 8,0	Clayfield (1974)
7.7 - 7.8	Halling-Sørensen and Hjuler (1992)

Timmermann and Van Hauten (1983) also showed the methanol/nitrate-N ratio as a function of pH. At optimum pH (=8,3 proposed by Timmermann *et al.*1983) the methanol/ nitrate-N ratio was found to be 2,52 g CH_3OH / NO_3^- - N, (Fig 4.6).

4.13 Combined Kinetic Expression for the Denitrification Process

As for the nitrification process, a combined expression for the denitrifer growth μ_D and nitrate removal, taking some of the environmental factors into account, can be formulated.

$$\mu_D = \mu_{max,D} * \frac{S_{denit}}{K_D + S_{denit}} * \frac{M}{K_M + M} \tag{4.33}$$

Removal rates can be related to growth rates through equation (4.34).

$$\frac{dS_{denit}}{dt} = -\frac{\mu_{max,D}}{Y_D} * \frac{S_{denit}}{K_D + S_{denit}} * \frac{M}{K_M + M} * X_D \tag{4.34}$$

Timmermanns and Van Hauten (1983) proposed an equation similar to (4.34), that also takes the influence of pH and temperature into account:

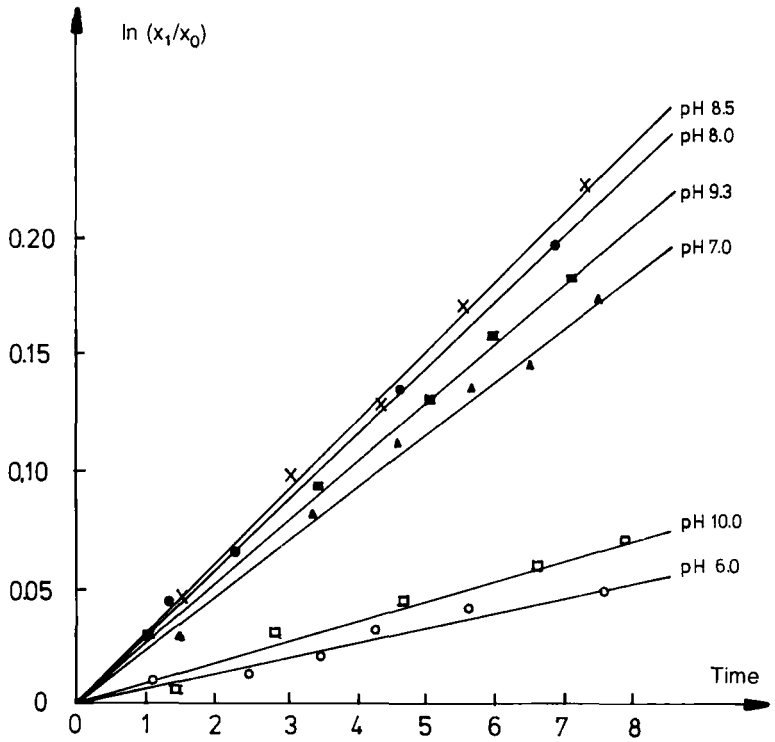

Figure 4.5 Determination of the growth rate μ at different pH values in a batch reactor at 25 °C. The biomass, X, is measured as a MLVSS concentration. After Timmermann and Van Hauten (1983).

$$\frac{dS_{denit}}{dt} = \frac{-\mu_{max,D}}{Y_D} * \frac{S_{denit}}{K_D + S_{denit}} * \frac{M}{K_M + M} * f[pH] * f[t] * X_D \qquad (4.35)$$

Assuming zero order kinetics, the equation proposed by Timmermann *et al.* (1983) can be rewritten as:

$$\frac{dS_{denit}}{dt} = -\frac{\mu_{max,D}}{Y_D} * f[pH] * f[t] * X_D \qquad (4.36)$$

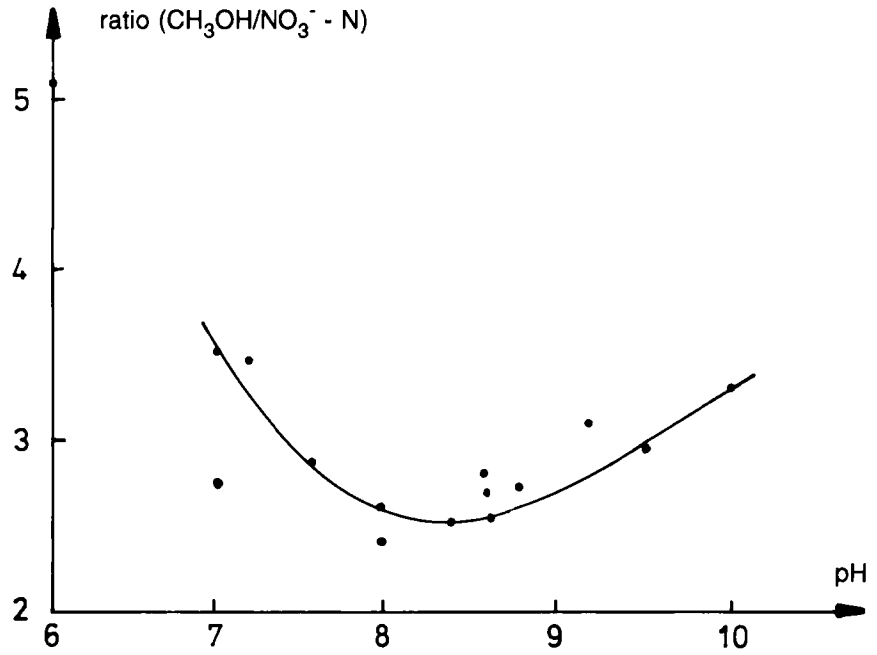

Figure 4.6 Methanol / nitrate-N ratio as a function of pH for the denitrification process. After Timmermann and Van Hauten (1983).

4.14 Bacterial Population Dynamics for the Denitrification Bacteria

The population dynamics of the denitrifying bacteria resemble the dynamics proposed for the nitrification bacteria, but the growth rate for the denitrifying bacteria is larger than for the nitrifying bacteria. It is, therefore not difficult for the denitrifying bacteria to compete with oxidizing bacteria in a combined organic and nitrogen removal, as is the case for the nitrifying bacteria.

The safety factor SF concept used in Section 3.12 can also be applied to denitrification. It can be related to nitrate removal rates through the following equation:

$$SF = \frac{\phi_d}{\phi_m} \tag{4.37}$$

where:

ϕ_d = solids retention time for the denitrification process

ϕ_m = minimum solids retention time for the denitrification process

In the case of denitrification, the safety factor can be related to nitrate removal rates, using the following two equations:

$$\frac{1}{\phi_d} = \mu_D - K_d \tag{4.38}$$

$$\frac{1}{\phi_m} = \mu_{max} - K_d \tag{4.39}$$

4.15 Influence of Toxic Substances on the Denitrification Process

The inhibition equation of the denitrification process resembles the equation proposed for the nitrification process in Section 3.13.

As for nitrification, the following overall expression takes both toxic substances and oxygen inhibition into account:

$$\frac{dS_{denit}}{dt} = -\frac{\mu_{max,D}}{Y_D} * \frac{S_{denit}}{K_D + S_{denit}} * \frac{M}{K_M + M} * f[I] * f[O_2] * X_D \tag{4.40}$$

where f[I] is a term taking the inhibition of toxic substances into account, and f[O_2] the oxygen inhibition, during the denitrification.

The major influence of toxic substances on denitrification is the short-term influence on the growth rate. It is of great importance that the denitrifying population is capable of dealing with different toxics, because then a long-term influence of the same toxic will not be as persistent as the short-term influence, since a bacteria population is very adaptive to all every environmental changes.
Many of the results referred to in the literature show how a short-term response can influence a population of bacteria, and may, therefore, often appear to be much more dramatic than a long-term influence, where the bacteria would have had time to adapt.

4.16 Conclusion

Chapters 3 and 4 summarize the results from many scientists concerning different factors affecting nitrification and denitrification.

It is often difficult in practice to evaluate the relevance of the different results, and thus it is also difficult to select the appropriate results for the planning of a particular biological nitrogen removal unit. The authors therefore recommend considering as many as possible of the different results mentioned, for the case study at the planning stage of a particular plant.

For example, Sections 3.8 and 4.12 give an overview of the influence of temperature on nitrification and denitrification processes, and refer to the results of numerous investigators. The various equations proposed should be tried in turn to see how they fit the case study, in order to avoid dimensioning errors in the completed unit. This approach, in effect, brings a safety factor into the plant design.