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Biological Removal of Nitrogen

Various measures have been taken over the last two decades by administrative authorities of many countries in the attempt to combat the increasing pollution of surface waters, a significant consequence of which can be eutrophication.

> Eutrophication is enrichment of nutrients in waters that stimulates an array of symptomatic changes, which can include increased phytoplankton and rooted aquatic plant production, fisheries and water quality deterioration, and other undesirable changes that interfere with water uses. (Pannard et al. 2017)

Eutrophication is an extremely complex phenomenon but can be described schematically: wastewater containing large quantities of nutrients such as nitrogen and phosphorus, when discharged into receiving environments, promotes the rapid and continuous growth of algae and aquatic plants.

Algae have a very short lifespan and decompose rapidly. This organic decomposition results in a high oxygen demand in the environment. The oxygen deficit creates anaerobic conditions, the initial consequences of which are the formation of hydrogen sulfide, ammonia, mineral matter, etc., which gradually clog the receiving environment where wastewater is discharged.

Of the nutrients responsible for these phenomena, nitrogen and phosphorus are the most detrimental. The two main methods of controlling eutrophication are the removal of nitrogen and the removal of phosphorus.

1.1. Nitrogen sources

Nitrogen is present in the air, soil and water in various forms, the chemical and biological combinations of which lead to very different reaction products, allowing us to deduce the cycle shown in Figure 1.1.

Nitrogen is found in mineral and organic forms. Mineral nitrogen is primarily in the form of ammonium ions (NH_4^+), nitrite ions (NO_2^-) and nitrate ions (NO_3^-). Organic nitrogen is present in the form of proteins which, through hydrolysis, form amino acids which in turn, through condensation, form peptides and polypeptides. It is also found in a wide variety of other organic compounds (urea, uric acid, creatinine, etc.).

Nitrogen removal presents serious ecological challenges for receiving water bodies, including eutrophication and toxicity to aquatic life due to its dissolved form as various compounds (Wiesmann 1994; Wang et al. 2017).

When organic matter is broken down by microorganisms, organic nitrogen is transformed into NH_4^+. The main wastewater concentrations of NH_4^+ and organic nitrogen are approximately 40 and 20 mg $N.L^{-1}$, respectively (Wiesmann 1994).

Nutrients contained in all kinds of organic matter can be recycled in various forms. Livestock effluents, crop residues and other organic by-products of human activities are a large resource for fertilization. Only prokaryotic organisms can reduce nitrogen into an assimilable recombinant form. Rhizobia belong to this group. The most efficient nitrogen-fixing systems are symbioses that couple nitrogen fixation with photosynthesis.

Ammonia synthesis uses natural gas and water to produce hydrogen gas (H_2) and combines it with nitrogen (N_2) present in the atmosphere to form ammonia (NH_3), a key ingredient in major nitrogen fertilizers.

Nitrogen fixation is the conversion of atmospheric nitrogen into nitrogen usable by plants and animals. This is carried out by certain bacteria that live in soils or water and can assimilate diatomic nitrogen N_2 and use it to synthesize their proteins.

Nitrogen reduction leads to the formation of diimine, and its oxidation leads to nitrous oxide (N_2O).

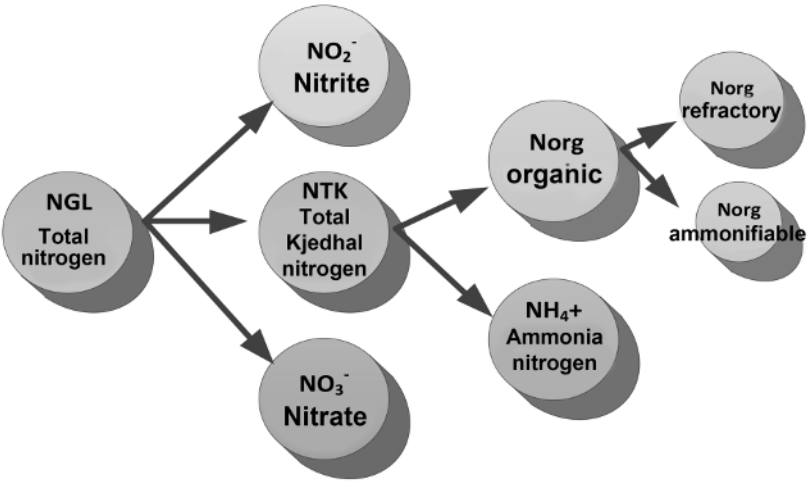


Figure 1.1. *Forms of nitrogen in wastewater*

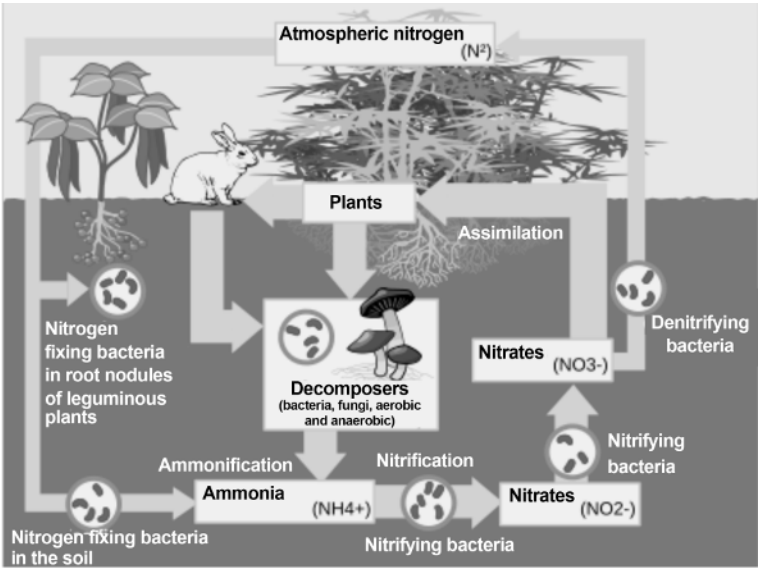
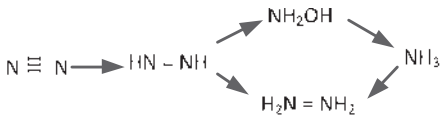
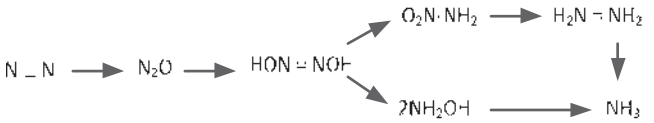


Figure 1.2. *Nitrogen cycle*



a)



b)

Figure 1.3. *a) Diimine. b) N_2O as intermediary*

Nitrogen fertilizer can contain nitrogen in the form of urea, ammonia, nitrate or a mixture of these forms. It is available in liquid form (nitrogen solution) or granular form (urea, ammonium nitrate).

1.2. Biochemical mechanisms of nitrogen utilization

Microbial organization transforms mineral nitrogen into organic matter. The activity of soil bacteria is primarily stimulated by ammonium. Organic nitrogen is not directly assimilable by plants; it must first be mineralized. Mineralization is the transformation of organic matter that leads to the formation of mineral salts, where fertilizing elements become soluble and accessible to plants. The mineralization of soil organic matter (and effluents) produces ammonium. The hydrolysis of urea by soil enzymes converts urea into ammonium and CO_2. Depending on the temperature, hydrolysis can take from one day to one week.

1.2.1. *Ammonification*

Wastewater entering a treatment plant contains a large proportion of organic nitrogen (albumin, carbamide). Furthermore, nitrogen discharged from residential settings is initially slightly more concentrated in ammonifiable organic nitrogen.

Organic nitrogen is said to be ammonifiable when it can be transformed by enzymatic hydrolysis into ammoniacal nitrogen. In other words, the ammonifiable/ammoniacal ratio can depend on factors such as temperature

and incubation time (depending on the length of the network) because there is a certain point that organic nitrogen is transformed into $N\text{-}NH_4^+$.

During treatment in a wastewater treatment plant, ammonification continues until the majority of the nitrogen is transformed into $N\text{-}NH_4^+$.

Soluble refractory nitrogen is the non-biodegradable component of nitrogen. Furthermore, this component is already detected upon entering the treatment plant. However, the quantity of this nitrogen in standard urban wastewater ranges from 1.5 to 2.5 $mg.L^{-1}$. Hard or refractory particulate nitrogen has similar concentrations but will be trapped in sludge. In some cases, the concentration of soluble hard nitrogen may be higher. This is generally due to sludge return or a system with a long retention time.

Ammonification involves nitrogenous substances of very diverse structures: proteins, amino sugars, nucleic acids, purine bases, amides, phosphatides, amines, uric acid and urea. The ammonia released from a protein substance corresponds to the following formula:

$N(NH_3)$ = N protein – (NH_3 metabolized by heterotrophs + NH_3 oxidized by autotrophs)

Ammonium is absorbed by roots more slowly than nitrate. This is particularly true for cyanobacteria and certain bacteria living in symbiosis with plants (including legumes). The typical chemical reaction is:

$$2\,N_2\,(g) + 3\,CH_2O + 3\,H_2O \rightarrow 4\,NH_4^+ + 3\,CO_2$$

nitrogen (gas) — *organic matter* — *water* — *ammonia* — *carbon dioxide*

In soils with a high pH, ammonium is transformed into ammonia:

$$NH_4^+ + OH^- \rightarrow NH_3\,(g) \rightarrow H_2O$$

ammonium — *hydroxide* — *ammonia (gas)* — *water*

The reaction requires an energy input from photosynthesis (cyanobacteria and legume symbionts). This fixation tends to produce ammonia compounds such as ammonium NH_4^+ and its conjugate acid, ammonia NH_3. This is a reduction reaction that takes place via organic substances denoted $\{CH_2O\}$.

Positively charged ammonium (NH_4^+) is adsorbed into the soil (soil cation exchange capacity (CEC)), which exchanges it with the roots. Most of the ammonium is converted to nitrate before being absorbed by plants.

Nitrate is rapidly absorbed due to its high mobility in soil water solution. Most annual crops preferentially use nitrate over ammonium.

Ammonification is the transformation of organic nitrogen into ammoniacal nitrogen. This reaction largely takes place in the sewer system upstream of the wastewater treatment plant.

The residual organic nitrogen is removed from the plant in various ways: part of it is trapped by sedimentation in the primary treatment zone, another part by filtration in the biocarbon filter, and the remainder is transformed into ammoniacal nitrogen as it flows through the treatment plant, mainly at the primary settling point. Generally speaking, almost all organic nitrogen is removed downstream of the plant.

Assimilation occurs during the biological elimination of carbon pollution by bacterial cells, mobilizing nitrogen.

When microorganisms cease their biological activity, the lysis of bacterial membranes provides a source of organic matter hydrolyzed into carbon and nitrogen. Heterotrophic biomass, by far the most significant contributor, plays a crucial role in producing these mineral elements, the quantities of which are proportional to its concentration.

The mineralization of living biomass leads to 0.09 g $N.g^{-1}$ COD eliminated; that is, 0.12 g $N.g^{-1}$ MVS eliminated:

$$C_5H_7NO_2 + 5\ O_2 \rightarrow 5\ CO_2 + NH_3 + 2\ H_2O$$

To remove nitrogen from wastewater, it is necessary to oxidize ammonium to nitrate, which must then be reduced to nitrogen gas. The nitrogen gas can then be permanently removed from the wastewater.

The oxidation of ammonium to nitrate is called nitrification. Nitrification by soil bacteria transforms ammonium into nitrate within a period ranging from a few days to a few weeks. Losses in the form of nitrous oxide or nitrogen oxide may occur during this process.

1.2.2. *Nitrification*

Nitrification transforms the products of fixation (NH_4^+, NH_3) into NO_x (NO_2^- and NO_3^-), nitrites and nitrates.

This is an oxidation reaction catalyzed by bacteria in soils and water. The biology of nitrifiers is dominated by their autotrophic nature, and they derive all their energy from oxidation reactions. This energy allows them to reduce carbon dioxide and use the carbon it contains. They are completely independent of any organic matter.

Nitrifying bacteria are classified into two categories:

– *Nitrosomonas*, which oxidize ammonia into nitrites;

– *Nitrobacters*, which oxidize nitrites into nitrates.

Each genus is specific in its reactions, and together they carry out nitrification. There are some heterotrophs such as *Aspergillus flavus* which oxidize NH_4^+ not only into nitrites but also into nitrates. However, their activity is less significant than that of nitrifying bacteria.

Nitritation is an oxidation reaction that produces:

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$$

$$2\ NH_4^+ + 3O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 4\ H^+$$

The formation of bacterial cells is governed by the following reaction:

$$5\ CO_2 + NH_4^+ + 2H_2O \rightarrow C_5H_7NO_2 + 5\ O_2 + H^+$$

Combining these two equations allows us to write the general equation:

$$55\ NH_4^+ + 5C0_2 + 760_2 \rightarrow C_5H_7NO_2 + 54\ NO_2 + 52H_2O + 109H^+$$

The nitration reaction involves Nitrobacter bacteria, which oxidize nitrites to nitrates according to the following reaction mechanism:

$$NO_2^- \rightarrow NO_2 + e^-$$

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + e^-$$

$$1/O_2 + 2\ H^+ + 2e^- \rightarrow H_2O$$

$$NO_2^- + 1/2O_2 \rightarrow NO_3^-$$

Combining the equations allows us to write the general equation:

$$400\ NO_2^- + 5\ CO_2 + NH_4^+ + 195\ O_2 + 2\ H_2O \rightarrow C_5H_7NO_2 + 400\ NO_3^- + H^+$$

The process is a two-step process. The reactions are as follows:

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^-$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O$$

Under specific conditions, some bacteria are capable of carrying out both of the above reactions. In the general context of wastewater and the conditions under which the processes are implemented, nitrification results from the sequential action of two groups of nitrifying organisms. These groups of nitrifying bacteria are presented in Table 1.1. *Nitrosomonas* and *Nitrobacter* are normally designated as the main nitrifying bacteria in wastewater treatment

Oxidation of ammonia	Nitrite oxidation
Nitrosomonas	*Nitrobacter*
Nitrococcus	*Nitrospina*
Nitrospira	*Nitrococcus*
Nitrosolobus	*Nitrospira*
Nitrosovibrio	

Table 1.1. *Some bacteria responsible for nitrification*

Carbon dioxide is used as a carbon source to produce new cellular tissue. In other words, they are chemolithotrophs. Taking into account the production of cellular tissue, the overall reaction for the nitrification process becomes:

$$NH_4^+ + 1.86O_2 + 1.98HCO_3^- \rightarrow 0.020C_5H_7NO_2 + 0.98NO_3^- + 1.04H_2O$$

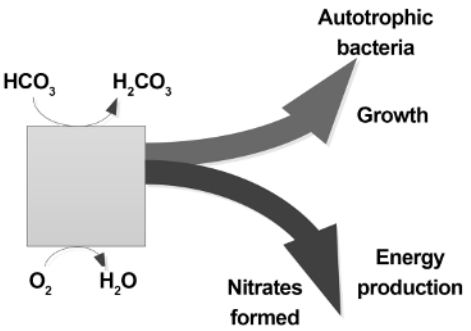


Figure 1.4. *Nitrogen utilization during assimilation (Spanjers 1996)*

This reaction shows that the nitrification process consumes both oxygen and alkalinity. The consumption of alkalinity means a decrease in pH. A significant decrease in pH will inhibit the process. An alkalinity of approximately 4 to 5 meq.L^{-1} in wastewater is often necessary to support the nitrification process.

The nitrification of 20 mg.L^{-1} d'N-NH_3 requires 84 mg.L^{-1} dissolved oxygen and produces 3 mg.L^{-1} with *Nitrosomonas*, 0.5 mg.L^{-1} with Nitrobacter and 2 moles of H^+ per mole of oxidized ammoniacal nitrogen.

1.2.3. *Denitrification*

The process of reducing nitrites to nitrogen is called denitrification. Wastewater treatment plants can be designed with a variety of features combining nitrification and denitrification, depending on the desired level of removal. Denitrification occurs when microorganisms lack oxygen (stagnant water and compacted soil).

During this process, soil bacteria convert nitrates (and nitrites) into nitrogen gas (N_2) and, to a lesser extent, nitrous oxide (N_2O) and nitrogen oxides (NO_X), which are released into the atmosphere. Denitrification can be carried out by autotrophs and heterotrophs.

1.2.3.1. *Heterotrophic denitrification*

Denitrification returns nitrogen to the atmosphere in its molecular form N_2, with CO_2 as a by-product of nitrogen oxide N_2O, a greenhouse gas that contributes to the destruction of the ozone layer in the stratosphere. This is a reduction reaction of NO_3^- through bacteria that transform organic matter.

The reaction is as follows:

$$4NO_3^- + 5CH_2O + 4H^+ \rightarrow 2N_2\,(g) + 5CO_2\,(g) + 7H_2O$$

Two mechanisms of nitrate metabolism are possible: the dissimilatory pathway or the ammonification of nitrates:

$$NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+ \text{ (by reductases)}$$

This reaction takes place in anaerobic habitats such as anoxic sediments and sludge, through strict or facultative anaerobic bacteria.

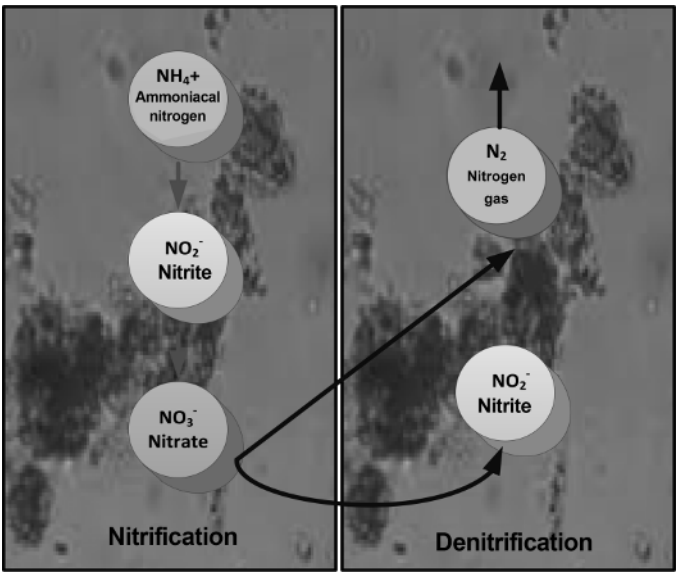


Figure 1.5. *Principle of biological elimination of mineral nitrogen*

Under certain conditions, microorganisms use nitrates as an oxidant instead of oxygen. The nitrates are then reduced to nitrogen and nitrogen oxides.

The goal of biological denitrification is therefore to completely remove nitrogen from wastewater. During this treatment process, nitrogen evaporates into the atmosphere in its molecular form, N_2. Denitrification is an anaerobic mechanism that allows a large number of heterotrophic bacteria to meet their energy needs using nitrates when dissolved oxygen is lacking. Without O_2, these bacteria must use the oxygen contained in nitrates for their respiratory needs.

The bacteria involved in the denitrification cycle are also involved in carbon alteration. In wastewater treatment plants, it is common to add an external carbon source (methanol, acetic acid, untreated sewage, industrial effluent) to compensate for carbon deficiencies.

Another advantage of denitrification is the recovery of alkalinity (a portion of that lost during the nitrification step). In fact, denitrification restores alkalinity equal to half the amount consumed by nitrification. That is, 1 kg of denitrified nitric nitrogen is equivalent to the addition of 1.95 kg of quicklime (CaO).

Furthermore, it is beneficial to implement a recirculation system in the water treatment process to reuse this alkalinity.

In conventional systems, biomass (activated sludge) is exposed to different environmental conditions to achieve COD and nitrogen removal.

Specifically, aerobic conditions are required for nitrification, while a certain amount of organic carbon compounds and the absence of oxygen are necessary to support denitrification.

The genera *Pseudomonas fluorescens* and *Bacillus* are the most common bacteria involved in heterotrophic denitrification. However, bacteria from other genera are possible: *Achromobacter*, *Aerobacter*, *Bacillus*, *Micrococcus*, *Paracoccus*.

Achromobacter	*Escherichia*	*Paracoccus*
Acinetobacter	*Flavobacterium*	*Propionibacterium*
Agrobacterium	*Gluconobacter*	*Pseudomonas*
Alcaligens	*Holobacterium*	*Rhizobium*
Bacillus	*Hyphomicrobium*	*Rhodopseudomonas*
Chromobacterium	*Kingella*	*Spirillum*
Cornybacterium	*Methanonas*	*Thiobacillus*
Denitrobacillus	*Moraxella*	*Xanthomonas*
Enterobacter	*Neisseria*	

Table 1.2. *Non-exhaustive list of heterotrophic bacteria in activated sludge (Ni et al. 2016)*

Numerous genera of bacteria, including heterotrophic denitrifying species, have been identified in activated sludge (see Table 1.2). Depending on the characteristics of the untreated water, treatment conditions and operating conditions, heterotrophic denitrifying bacteria represent approximately 50 to 80% of all flocculated and dispersed bacteria in activated sludge. Most of these denitrifying bacteria are facultative aerobic bacteria that prefer oxygen molecules to nitrogen oxide molecules as electron acceptors. Therefore, the absence of oxygen or the presence of an oxygen gradient within the activated sludge is necessary for efficient heterotrophic denitrification.

In this type of process, the source of organic carbon is fundamental: it is available in food and animal or plant waste. In the field of wastewater treatment, a carbon source is provided by organic carbon (BOD) already present in wastewater or through the addition of acetic acid, methanol or ethanol. These facultative anaerobic bacteria are capable of reducing nitrate and nitrite to nitrogen gas under anaerobic conditions. These microorganisms use either oxygen or oxidized forms of nitrogen as final electron acceptors.

With too little carbon, the reaction is incomplete and nitrites appear, while too much carbon promotes an overly reducing environment, which leads to the production of sulfides (rotten egg smell).

Industrial effluents laden with organic matter, such as those from refined sugar, fruit juice or even dairy products, can aid in denitrification.

The fundamental reaction in the presence of organic matter in wastewater is written as:

$$0.61.\ C_{18}H_{19}O_9N + 4.5\ NO_3^- + 0.39\ NH_4^+ + 4.15\ H_3O^+ \rightarrow C_5H_7NO_2 + 2.27\ N_2 + 5.98\ CO_2 + 5.15\ H_2O$$

This reaction of organic matter degradation, all other conditions being equal, is slower than that which occurs in the presence of oxygen. It is even slower the less readily biodegradable the available carbon.

The fundamental reactions of denitrification in the presence of methanol are:

– Required energy:

$$2CH_3OH + 6\ NO_3^- \rightarrow 2CO_2 + 4\ H_2O + 6\ NO_2^-$$

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

i.e.:

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

– Cell synthesis:

$$100\ CH_3OH + 97\ NO_3^- \rightarrow 5\ C_5H_7NO_2 + 75\ CO_2 + 134\ H_2O + 46\ N_2 + 97\ OH^-$$

– Endogenous respiration:

$$5C_5H_7NO_2 + 23NO_3^- \rightarrow 25CO_2 + 6H_2O + 14N_2 + 23OH^-$$

Factors affecting denitrification are:

– Organic carbon: denitrifying bacteria can use different types of organic matter as carbon and energy sources. Organic matter in wastewater, the addition of methanol, ethanol, acetic acid or other organic compounds, can be used for denitrification. In biological denitrification, it is important to estimate the mass of COD required to reduce nitrates. Generally, approximately 3.5–4 g of biodegradable organic carbon are needed per gram of reduced NO_3^-. However, this ratio depends largely on the nature of the carbon and the microbial ecology. Generally speaking, since

denitrifying bacteria grow with high cell yields (am), a high COD/N ratio is required.

– Temperature: the denitrification process is temperature-dependent, with the denitrification rate increasing at higher temperatures. The reported optimal temperature varies from 20 to 35°C. As the growth rate of denitrifying agents decreases with a drop in wastewater temperature, this leads to a reduction in denitrification capacity during winter. Denitrification is considerably inhibited at wastewater temperatures below 5°C.

– Oxygen: oxygen inhibits the denitrification process, which occurs under anoxic conditions. If oxygen is present, bacteria will use molecular oxygen instead of that from nitrates and nitrites as an electron acceptor. It is important to note that the actual oxygen concentration experienced by bacteria within activated sludge flocs or biofilms may be lower than the overall dissolved oxygen concentration that is measured. An overall concentration lower than 1 mg $O_2.L^{-1}$ is often recommended. However, even if a very limited amount of oxygen is introduced into the denitrification tank (DN), it is immediately consumed by the denitrifying bacteria. Denitrification can therefore still occur with a limited concentration of O_2. In an activated sludge system, bacteria group together, forming sludge flocs. Within biofilms, oxygen diffuses throughout the floc, creating an oxygen gradient. This allows denitrification to occur in the internal zones of the flocs.

– pH and alkalinity: the optimal pH for denitrification is between 7 and 9, with the denitrification rate decreasing sharply outside this range. In most cases, the pH is stable between 7 and 8 in biological systems, suggesting that pH should only slightly affect denitrification. The denitrification process produces alkalinity: approximately 0.07 meq of alkalinity per mg of reduced $N\text{-}NO_3$. An optimal pH is between 7 and 9.

– Nutrients: nutrient requirements are identical to those of other heterotrophic microorganisms. Phosphorus can be a limiting factor if precipitation is used prior to the biological process.

1.2.3.2. *Autotrophic denitrification*

Autotrophic denitrifying bacteria are capable of reducing oxidized forms of nitrogen, using carbon dioxide or bicarbonate.

A very wide diversity of bacterial genera are capable of denitrification; several classes of bacteria include:

– Chemolithoautotrophs and heterotrophs that use organic carbon.

– Chemolithoautotrophs, capable of oxidizing elemental sulfur (S°) and other sulfur compounds such as sulfites (SO_3^{2-}), sulfides (S^{2-}) and thiosulfate ($S_2O_3^{2-}$), which can be classed into two groups:

- Obligate chemolithoautotrophic bacteria that can only be used with sulfur, such as *Thiobacillus denitrificans*, *Thiomicrospira denitrificans* and *Sulfurimonas denitrificans*. These reactions involve denitrification processes that occur in the presence of sulfur compounds. They are practically limited to autotrophic growth, as they can only obtain energy by oxidizing sulfur compounds (Kuenen and Robertson 1992).

- Facultative chemolithoautotrophic bacteria, such as *Thiobacillus versutus*, *Thiobacillus thyasiris*, *Paracoccus denitrificans* and *Thiosphaera pantotropha*, which use sulfur as an energy source but can also grow heterotrophically by deriving their energy from an organic source. Autotrophic bacteria can oxidize an inorganic substrate such as sulfur; therefore, granular sulfur is used as a bacterial substrate. This produces energy used by microorganisms for growth. During autotrophic denitrification with elemental sulfur, the carbon source that the bacteria consume is carbon dioxide. This type of system does not require external intervention to enrich the environment with a carbon source; it is completely self-sustaining.

Autotrophic denitrification primarily results in sulfates with the production of a H^+ ion as its end product: sulfuric acid is generated, which lowers the pH (6.0 to 6.5).

The use of *Thiobacillus denitrificans* can allow the reduction of nitrates to nitrogen through a variety of sulfur compounds (S^{2-}, S, $S_2O_3^{2-}$, $S_4O_6^{2-}$, SO_3^{2-}) as they are oxidized into sulfates. Sulfur is a constituent of living matter. It makes up approximately 1% of the cell's dry weight and is a component of amino acids; for example, in the form of –SH groups (cysteine), disulfides (cystine) or thioethers (methionine). It is a vital element of coenzymes (thiamine, biotin, lipoic acid). It is present in the cartilage of higher animals (chondroitin sulfuric acid), in various natural products produced by plants, in certain antibiotics (penicillin) and in nature primarily in the form of sulfates.

Generally speaking, sulfur can be present in the form of elemental sulfur, sulfides, disulfides, sulfates, thiosulfates and even thiocyanates. Its use depends on its state. Sulfur is assimilated by animals and plants in the form of sulfates, which are extremely abundant in nature.

For example, Winogradsky (1890) showed that cell cytoplasm granules contain colloidal elemental sulfur. This sulfur, accumulated inside the bacterium, comes from the oxidation of sulfides in the environment. When cells lack sulfides, they oxidize the cytoplasmic colloidal sulfur into thiosulfates, sulfites and sulfates, which are then transformed into sulfides by other bacterial species.

As with nitrifying bacteria in the nitrogen cycle, these bacteria derive their energy from the oxidation of reduced sulfur compounds. Sulfate-reducing bacteria assimilate sulfates, transforming them into sulfides. These sulfides are then assimilated by sulfate-oxidizing bacteria, which transform them back into sulfates.

This establishes an equilibrium between the two bacterial flora, which cycle sulfur from its reduced to its oxidized state and vice versa.

1.2.3.2.1. Sulfur-oxidizing bacteria

There are many forms of sulfur-oxidizing bacteria, both morphologically and physiologically. Winogradsky showed that filamentous bacteria *Beggiatoa alba* oxidize sulfides aerobically in two steps. The first step is the oxidation of sulfides to elemental sulfur, which accumulates in the cytoplasm in the form of granules:

$$H_2S + ½\ O_2 \rightarrow S° + H_2O \quad \Delta G° = 171.4\ kJ.mole^{-1}$$

When the sulfide is depleted, the elemental sulfur granules are oxidized into sulfates:

$$S° + 3/2\ O_2 + 3H_2O \rightarrow SO_4^{2-} + 2H_3O^+ \quad \Delta G° = -493.2\ kJ.mole^{-1}$$

These bacteria use CO_2 as the sole source of carbon. Other types of bacteria, like *Thiobacillus*, are responsible for oxidation into sulfates, sulfides, thiosulfates, elemental sulfur and thiocyanates. Various species exist depending on whether the environment is aerobic or anaerobic.

In aerobic conditions, the reactions are as follows:

– With *Thiobacillus thioxidans*:

$$4S° + 6O_2 + 12H_2O \rightarrow 4SO_4^{2-} + 8H_3O^+ \quad \Delta G° = -575.1\ kJ.mole^{-1}$$

– With Thiobacillus thioparus:

$$H_2S + 2O_2 + 2H_2O \rightarrow SO_4^{2-} + 2H_3O^+ \quad \Delta G° = -780.4\ kJ.mole^{-1}$$

– With Thiobacillus thioparus:

$$S_2O_3^{2-} + 2O_2 + 3H_2O \rightarrow 2SO_4^{2-} + 2H_3O^+ \quad \Delta G° = -1\ 060\ kJ.mole^{-1}$$

The stoichiometries of some denitrification reactions using sulfur compounds as an energy source are given below:

$$0.844.\ S_2O_3^{2-} + NO_3^- + 0.347CO_2 + 0.086HCO_3^- + 0.086NH_4^+ + 0.434H_2O \rightarrow 1.689SO_4^{2-} + 0.500N_2 + 0.086C_5H_7O_2N + 0.697H^+$$

$$0.421H_2S + 0.421HS^- + NO_3^- + 0.346CO_2 + 0.086HCO_3^- + 0.086NH_4^+ \rightarrow 0.842SO_4^{2-} + 0.500N_2 + 0.086C_5H_7O_2N + 0.434H_2O + 0.262H^+$$

$$1.10.\ So + NO_3^- + 0.76H_2O + 0.40CO_2 + 0.08NH_4^+ \rightarrow 1.10SO_4^{2-} + 0.50N_2 + 0.08C_5H_7O_2N + 1.28H^+$$

Thiobacillus are spore-forming, rod-shaped Gram-negative bacteria, with sizes between 1 and 3 µm. Beijerinck (1910) showed that *Thiobacillus thioparus* develop at a pH close to neutral and their growth ceases around pH 5. However, *Thiobacillus thioxidans* only develop at pH 5 and their growth can lower the pH to 0.

In anaerobic conditions, anaerobic facultative *Thiobacillus* use nitrates as an oxygen source, generally *Thiobacillus denitrificans*.

The equation is as follows:

$$5S° + 6NO_3^- + 6H_2O \rightarrow 5SO_4^{2-} + 4H_3O^+ + 3N_2$$

Beijerinck (1910) showed that the optimum pH for the growth of these bacteria was between 6.2 and 7.4.

1.2.3.2.2. Sulfate-reducing bacteria

Desulfovibrio desulfuricans have often been identified as sulfur-reducing bacteria. These are non-spore-forming, Gram-negative, highly motile bacteria, between 1 and 2 µm in size, and are strict anaerobes. They reduce sulfates to sulfides using organic aromatic compounds such as dicarboxylic acids, pyruvates or lactates as an energy source.

1.3. Factors influencing nitrification

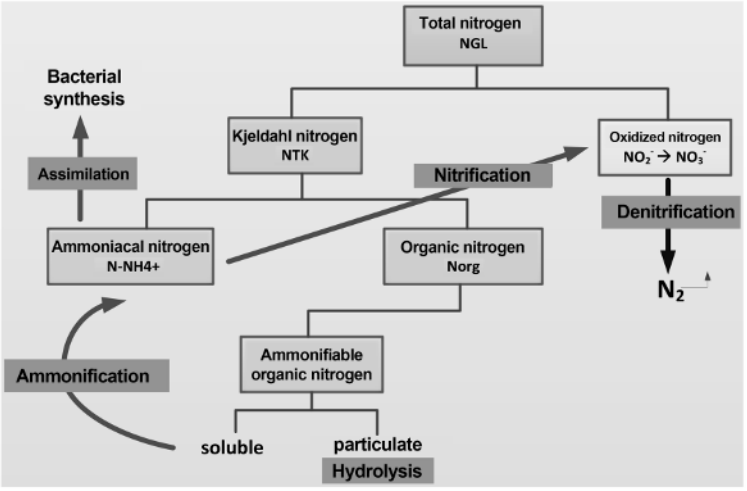


Figure 1.6. *Factors involved in nitrogen removal*

Human activity contributes to increased denitrification, among other things, through the use of fertilizers that add ammonia compounds to the soil (NH_4^+, NH_3) and nitrates (NO_3^-). The use of fossil fuels in engines or thermal power plants transforms nitrogen into nitrogen oxide NO_2^-. With N_2 and CO_2, denitrification releases a small amount of nitrogen oxide N_2O into the atmosphere. The concentration of this gas is low, 300 ppb (parts per billion). However, it should be noted that a molecule of N_2O is 200 times more effective than a molecule of CO_2 in creating a greenhouse effect. It is currently estimated that the concentration of atmospheric N_2O increases annually by 0.3%, and this increase is almost entirely linked to emissions due to soil denitrification. Studies of Antarctic ice cores have shown that the concentration of atmospheric N_2O was 270 ppb at the end of the last ice age (10,000 years ago). This concentration remained at that level until the industrial era, when it jumped to its current level of 300 ppb, an increase of 11%. For the nitrification process to be efficient, the concentration of organic matter must be low. Otherwise, fast-growing heterotrophic microorganisms will consume the oxygen necessary for their survival. Factors affecting the nitrification process include temperature, oxygen concentration, pH, alkalinity and inhibitory substances in wastewater.

1.3.1. *Temperature*

Nitrification is strongly affected by temperature; temperature alters the rate of synthesis of nitrifying bacteria below 30°C. Between 30 and 35°C, growth is little influenced by temperature, and it then becomes impossible beyond 35°C (see Figure 1.7).

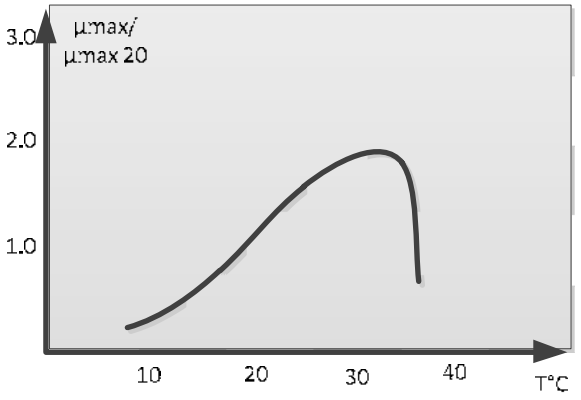


Figure 1.7. *Influence of temperature on the maximum growth rate of autotrophs (Henze et al. 1995)*

A drop in temperature from 16°C to 6°C will reduce the nitrification rate by 2.5 times. Temperature is an important parameter in all biological reactions and has a pronounced effect on the growth rate of nitrifying organisms.

1.3.2. *Growth rate*

The growth rate of autotrophic bacteria is given in Table 1.3.

Temperature (°C)	Growth rate	
	Maximum (μmax) (d^{-1})	Observed on site (d^{-1})
20	0.7	0.47
10	0.21–0.31	0.14–0.21

Table 1.3. *Growth rate of autotrophic bacteria*

COMMENTS ON TABLE 1.3.– *Maximum (μmax): obtained in the laboratory without limiting factors; observed on site: function of the maximum growth rate and a number of factors such as the concentration of N-NH_4^+, oxygen concentration, temperature and minimum oxygen presence.*

$$\mu\text{nit site} = \mu\text{nit max}\frac{NH_4}{K_{NH4}+NH_4}\ \frac{O_2}{KO_2+O_2}$$

where:

– μnit max = maximum growth rate of nitrifying bacteria (/t);

– NH_4 = NH_4 concentration in the interstitial fluid ($mg.L^{-1}$);

– K_{NH4} = [NH_4] for which μ = μnit max/2 ($mg.L^{-1}$);

– O_2 = concentration of dissolved oxygen in the interstitial fluid ($mg.L^{-1}$);

– K_{O2} = dissolved oxygen concentration for which μ = μnit max/2 ($mg.L^{-1}$).

Furthermore:

$$\mu\text{nit max (T)} = \mu\text{nit max (20°C)} \times \theta^{(T-20)}$$

With θ = 1.06, we obtain:

$$\mu\text{nit max (20°C)} = 0.8\ d^{-1}$$

At 10°C:

$$\mu\text{nit max (T)} = \mu\text{nit max (20°C)} \times \theta^{(T-20)}$$

$$\mu\text{nit max (10°C)} = 0.8 \times 1.06^{-10} = 0.45\ \text{d}^{-1}$$

The temperature change constant (θ) takes on quite different values depending on the author: 1.02 (Oleszkiewicz and Berquist 1988), 1.024 (Argaman 1994; Mines and Sherrard 1999), 1.044 (Funamizu and Takakuwa 1994), 1.06 (Deronzier et al. 2002), 1.103 (Lesouef 1992) and 1.165 (McCartney and Oleszkiewicz 1990).

Henze et al. (1995) propose the following:

$$\mu\text{n max(pH)} = \mu\text{n max(pH optimal)} \frac{\text{KpH}}{\text{KpH} + 10^{(\text{pHoptimal}-\text{pH})}}$$

with KpH = 200.

In winter, complete nitrification depends on a combination of several factors such as the volume of the aeration tank, suspended solids (SS) and pH, whereas in warmer periods, lower sludge levels and a less stringent pH value are sufficient.

1.3.3. *Oxygen*

Nitrifying bacteria are more sensitive to low oxygen concentrations than heterotrophic microorganisms. At O_2 concentrations below 2–3 $mg.L^{-1}$, this will likely be a limiting factor depending on the type of biological treatment used.

1.3.4. *pH and alkalinity*

The nitrification process is highly dependent on pH and alkalinity.

The optimal pH values for *Nitrosomonas* are located between 8 and 9, while at pH 7, their activity decreases by 50%. For *Nitrobacter*, the maximum growth rate occurs at pH 7–8.6.

The pH of the wastewater will determine the ammonia concentration (NH_3) and nitric acid (HNO_2), which in turn can inhibit nitrifying bacteria. The nitrification process also consumes alkalinity, which can lead to a reduction in pH.

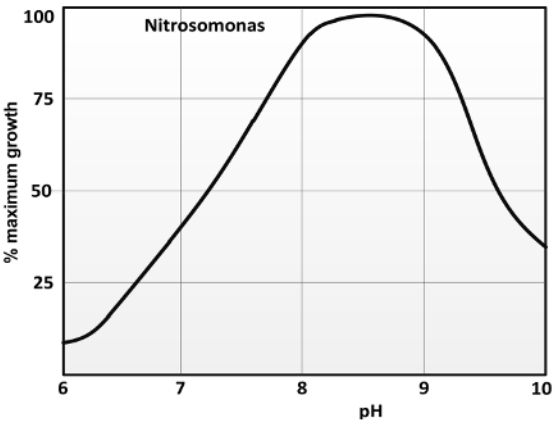


Figure 1.8. *Influence of pH on the growth of* Nitrosomonas *at 20°C (Wild 1994)*

1.3.5. *Nutrients*

In municipal wastewater, the amount of nutrients is normally sufficient for microbial growth. However, phosphorus can become a limiting factor when pre-precipitation is used upstream of the biological process. At ortho-P concentrations < 0.15–0.2 $mg.L^{-1}$, the nitrification reaction could be inhibited.

1.3.6. *Dissolved oxygen and nitrogen substrate*

Dissolved oxygen and ammoniacal nitrogen are the essential reactants for nitrification. When present in sufficient quantities, the growth of autotrophic bacteria is maximized. When one of the two reactants becomes limiting, bacteria have more difficulty accessing it, which slows their growth rate. Manufacturers typically describe the influence of dissolved oxygen and nitrogen substrate on the growth rate of autotrophs using a double Monod equation:

$$\mu aut(T) = \mu aut\,max(T)\frac{O_2}{O_2 + KO, aut}\frac{N - NH_4}{N - NH_4 + K_{NH4}}$$

Practical and easy to implement, this type of relationship is also used to predict the growth rate of heterotrophs during assimilation. In an oxygen-limited environment, autotrophic and heterotrophic biomasses compete. The course of the reactions depends on the bacteria's ability to access oxygen at low concentrations. Specific to each biomass, this affinity is expressed as a half-saturation constant.

Heterotrophic bacteria, which more readily use oxygen at low concentrations, are characterized by a lower half-saturation constant (KO, heterotrophs = 0.2 $mgO_2.L^{-1}$) than that of autotrophs (KO, autotrophs = 0.2 $mgO_2.L^{-1}$). The value of K_{NH4} is equal to 0.1 $mg.L^{-1}$ (Henze et al. 1986).

Thus, the main factors influencing the growth of autotrophic bacteria in activated sludge are pH and temperature, which affect the internal mechanisms of the bacteria. They determine the maximum growth rate of nitrifying biomass (μA, maxi), ammoniacal nitrogen and dissolved oxygen through their availability. Their concentrations modulate the maximum growth rate of autotrophic biomass.

Net growth, resulting from growth and death, taking into account both the limitation of the dissolved oxygen concentration and the nitrogen substrate, is then defined for a continuous 24 h oxygenation period by the equation:

$$\mu aut = \mu aut\, max(20°C)\, \Theta^{T-20} \frac{O_2}{O_2 + KO, aut} \frac{N - NH_4}{N - NH_4 + K_{NH4}} - baut$$

i.e.:

$$\mu aut = \mu aut\, max(20°C)\, \Theta^{T-20} \frac{O_2}{O_2 + 0.4} \frac{N - NH_4}{N - NH_4 + 0.1} - baut$$

If the oxygenation time is not continuous, this parameter is integrated and the equation becomes:

$$\mu aut = \mu aut\, max(20°C)\, \Theta^{T-20} \frac{O2}{O2 + 0.2} \frac{N - NH_4}{N - NH_4 + 0.1} \frac{t}{24} - baut$$

where t is the operating time of the aerators (h).

Table 1.4 summarizes the main growth parameters for the nitrogen treatment simulation.

Reference 10°C		Symbol	Units	Values
Maximum growth rate		μ max	d^{-1}	0.45
Saturation constant	**Substrate**	Ks NH_4	g $N\text{-}NH_4^+.m^{-3}$	0.1
	Oxygen	Ks O_2	g $O_2.m^{-3}$	0.2
Cellular yield of autotrophs		Y max	g $MVS.g^{-1}$ $N\text{-}NO_3$ formed	0.24
Endogenous respiration constant		b autotrophs	d^{-1}	0.13
Temperature constant for μ max		Θ	$°C^{-1}$	1.06

Table 1.4. *Growth parameters for autotrophic bacteria*

1.4. Nitrification in an activated sludge system

The implementation of nitrification in activated sludge depends on several factors that influence the sizing of the biological reactors.

1.4.1. *Influence of mass loading*

The mass loading corresponds to the quantity of BOD_5 applied daily to the biomass present in the system (kg of MVS). Mass loading has several consequences: on the plant's efficiency, on the sludge's volatile solids (VSS) content, on the culture age, on the specific sludge production and on oxygen consumption. As the mass loading (Cm) increases, the age of the sludge decreases. Indeed, the increase in organic matter leads to a significant increase in the sludge mass, and therefore in the mass to be extracted (Xa). The extractions gradually remove the autotrophic bacteria from the environment.

The classification of processes is established according to Table 1.5.

Achieving the classically accepted rejection level (COD < 125 $mg.L^{-1}$, BOD_5 < 25 $mg.L^{-1}$ and TSS < 35 $mg.L^{-1}$) requires operation at the mass loads shown in Table 1.6.

Mass load (kg BOD.kg.MVS^{-1}. d^{1})	**Process**
Cm < 0.1	Extended ventilation
0.1. < Cm < 0.2	Low load
0.2 < Cm < 0.5	Medium load
Cm > 0.5	High load
Cm > 1	Very high load

Table 1.5. *Classification of processes based on mass loading*

Mass load (kg BOD.kg.MVS^{-1}. d^{-1})	**Temperature**	**Process**
Cm ≤ 0.35	10°C	Medium load
Cm ≤ 0.8	20°C	Heavy load

Table 1.6. *Influence of temperature for constant NH_4 yield*

The influence of mass loading on nitrification is as follows:

– A high proportion of organic matter promotes the growth of heterotrophic bacteria.

– The cellular yield of autotrophs (*Nitrosomonas* and *Nitrobacter*) is much lower than that of heterotrophs. The autotrophic population declines rapidly due to sludge removal.

– For optimal nitrification to occur, it is necessary to establish a balance between heterotrophs and autotrophs; the decomposed organic matter will form CO_2 which will be used by autotrophs to synthesize new cells.

– The increase in mass loading causes oxygen deficiencies in the tank and affects nitrification.

Mass loadings less than 0.03–0.04 kg of $BOD_5.kg^{-1}$ MVS. d^{-1} are too low. This leads to the formation of small flocs due to slight deflocculation linked to a limited amount of substrate. These flocs result in lower sedimentation rates, which can negatively impact discharge during hydraulic surges in the clarifier. High mass loadings, in addition to the non-compliant yield (yield decreases with increasing mass loading), also lead to the formation of small flocs. Furthermore, sudden changes in mass loading (from a low to a high value) frequently result in the growth of filamentous bacteria. The mass loading remains fairly stable if the sludge content is kept constant by the operator (Canler et al. 2007).

1.4.2. *Influence of TSS/MVS concentration in the tank*

The concentration of SS has consequences for the sizing of the biological reactor. For the average load, an SS concentration of up to 3 g $MVS.L^{-1}$ is used, that is, a TSS rate of 4 $g.L^{-1}$. An excessively high SS level in the biological reactor, combined with typical recirculation rates of 150%, can cause system blockages due to limited thickening resulting from a degradation of the sludge index and trigger sludge removal (Canler et al. 2007). In the case of low loading rates or prolonged aeration, increasing the concentration in the aeration tank is more advantageous. It is possible to work with higher concentrations (4.5 $g.L^{-1}$).

1.4.3. *Influence of loading rate*

The volumetric charge or loading rate of the system is obtained from the ratio of the quantity of BOD_5 to be treated per day and the reactor volume or from the product of the Cm and the MVS concentration:

Loading rate (Cv) = mass load x MVS concentration

i.e.:

– Cv = flow rate BOD_5 (kg. d^{-1})/reactor volume (m^3);

– T retention time (hour or day) = reactor volume (m^3)/hourly or daily flow rate;

– Cv = BOD_5 concentration × 1/retention rate.

This volumetric loading rate therefore corresponds to the retention time of the wastewater to be treated with biomass in the biological reactor. We obtain a Cv value of less than 0.6 kg $BOD_5.m^{-3}. d^{-1}$.

1.4.4. *Influence of sludge age*

The sludge age is the ratio (G) of the mass of sludge present in the aerator (Xa) to the mass (Xb) of sludge removed daily. It is expressed in days, that is, G = Xa/Xb.

This quotient therefore represents the average retention time of the sludge in the active part of the system and is directly related to its state of evolution. Achieving a high sludge age to maintain autotrophic biomass requires working at a specific mass loading. The maximum mass loadings and minimum sludge ages required for the nitrification process as a function of temperature are presented in Table 1.7.

Temperature in the biological reactor	10°C	15°C	20°C
Mass load (kg of BOD_5.kg^{-1} of MVS. d^{-1})	0.1	0.15	0.2
Age of the sludge (days)	16–18	10–12	8–9

Table 1.7. *Relationships between temperature, mass loading and age of the sludge*

1.4.5. *Nitrification rate*

$$Vnit\ T = A\ Cv_N\ \theta^{(T-20)}$$

where:

– A constant = 0.1161 at 20°C;

– Vnit T = nitrification rate at temperature T (mg $N.L^{-1}.h^{-1}$);

– T = temperature (°C);

– θ = corrective coefficient = 1.06;

– C_{VN} = NTK volumetric load (g NTK input.m^{-3} in reactor. d^{-1}).

1.4.6. *O_2 requirement*

Under average load as well as during prolonged ventilation, it is necessary to cover the sum of the needs corresponding to:

– the oxidation of organic matter;

– the auto-oxidation of sludge (endogenous respiration);

– nitrogen oxidation (reduced by the oxygen release observed during denitrification).

Furthermore, the daily operating time of the aeration system must allow for periods of anoxia necessary for denitrification. These periods correspond to the difference in the amount of oxygen required to oxidize ammonia nitrogen to nitric nitrogen (nitrification) and the amount of oxygen recovered by the reduction of nitric nitrogen to nitrogen gas (denitrification), which can be written as:

$$NH_4OH + 2O_2 \rightarrow NO_3H + 2H_2O$$

– Consumption of 4.57 mg O_2.mg^{-1} N oxidized:

$$2NO_3H \rightarrow N_2 + H_2O + 5/2O_2$$

– Recovery of 2.86 mg O_2. mg^{-1} N reduced.

– Nitrification: oxygen requirements due to nitrification:

$O_2n = 4.57 \times$ N nitrified

– Denitrification: the amount of oxygen recovered by denitrification:

O_2 dn = 2.86 × N denitrified

1.4.6.1. *Nitrogen balance*

1.4.6.1.1. *Nitrogen for synthesis*

A quantity of nitrogen (in the form of TNK) is removed simultaneously with BOD and is quantitatively present in the sludge produced. The concentration of synthetic nitrogen is an estimated 5% of the removed BOD flow:

N synthesis (Nsy) = 5% BOD removed

1.4.6.1.2. Nitrogen removed by the nitrification–denitrification process

If nitrogen removal by synthesis alone is insufficient to meet the required nitrogen standard for the treated effluent, additional nitrogen must be removed by nitrification–denitrification. The calculation is performed retrospectively as follows:

Nitrified nitrogen (Nnit) = NTK influent – NTK effluent – Nsy ($kg.d^{-1}$)

Denitrified nitrogen (Ndenit) = N nitrified – $N.NO_3$ effluent ($kg.\ d^{-1}$)

These values are established by taking nitrogen standard values for NTK effluent and $N\text{-}NO_3$ effluent.

For example, for 10 $mg.L^{-1}$ NGL, NTK = 5 $mg.L^{-}1$ and $N\text{-}NO_3$ = 5 $mg.L^{-1}$. For 20 $mg.L^{-1}$ NGL, NTK = 10 $mg.L^{-}1$ and $N\text{-}NO_3$ = 10 $mg.L^{-1}$.

The nitrification objective is then considered to be achieved provided that a mass of MVS is maintained in aeration (in the aerobic zone) such that: nitrified nitrogen/aerobic $X \leq 3$ $mg.g^{-1}$ $MVS.h^{-1}$.

1.4.6.2. *Minimum sludge age required for nitrification*

The minimum sludge age required for nitrification is calculated based on the aerated volume and is a function of the temperature (T): this formula is valid for a treatment plant serving a population greater than 100,000 individuals:

$$Gnit = FS \times 2.13 \times 1.103^{(15-T)} \text{ in days}$$

FS = 2.3 for a plant treating a population greater than 100,000 individuals.

The minimum sludge age recommended for nitrification is typically around 8 days.

Oxygen requirements can also be defined in relation to the entering BOD_5. The total oxygen demand expressed relative to the BOD_5 applied is calculated from:

– Specific oxygen requirements due to the elimination of BOD_5 (O_{RBOD5}):

O_{RBOD5} ($kg\ O_2.kg^{-1}\ BOD_5$)

– Specific oxygen requirements due to nitrogen removal (O_{RN}):

O_{RN} ($kg\ O_2.kg^{-1}\ BOD_5$)

The specific needs (both for BOD_5 as with nitrogen) are defined in relation to the entering BOD_5:

$$O_2 \text{ (kg } O_2.d^{-1}) = Q\ (O_{RBOD5} + O_{RN})\ Lo/1{,}000$$

where Lo is the BOD_5 wastewater entering the biological reactor ($mg.L^{-1}$).

The assessment of specific needs for the BOD_5 and nitrogen is given by:

– The specific oxygen consumed by the BOD_5 (O_{RBOD5}): the oxygen consumed by carbon pollution depends on the age of the sludge and the temperature:

$$\text{ORBOD5} = \frac{0.144\ \text{G f}}{1 + 0.08\text{G f}} + 0.5$$

where O_{RBOD5} is in kg $O_2.kg^{-1}$ BOD_5, G is the sludge age (d) and f is the temperature correction factor.

– The oxygen demand specific to nitrification, taking into account the oxygen recovered by denitrification O_{RN}:

$$\text{ORN} = \frac{\text{Q}(4.6\ \text{N} - \text{NO3} - 1.7\ \text{N} + \text{NO3 denitrified})}{\text{Q x BOD5}}$$

where O_{RN} is in kg $O_2.kg^{-1}$; BOD_5 and N-NO_3 are in $kg.d^{-1}$, and Q is in $m^3.d^{-1}$.

1.4.7. *Dynamic model of nitrification*

If we assume complete mixing, that is, instantaneous mixing of the inlet water throughout the entire volume of the aeration tank, we can describe the nitrification process by performing mass balances on both the nitrogen removed and the sludge present. Consider an activated sludge treatment plant (see Figure 1.9) consisting of a biological reactor with volume V containing sludge with concentration Xa receiving an effluent of concentration Lo at a daily volume Q. To return the sludge from the settling tank to the reactor inlet, a recycling rate r is applied.

Part of the removed carbon pollution is transformed into biomass, most of which is extracted (concentration Xb with a flow rate σQ). The treated effluent obtained at the outlet has a soluble pollution (Lf) and SS (Xf) concentration. X denotes MES; X_V denotes MVS; X_N is the nitrifying mass; Xh denotes heterotrophic organisms; b is the endogenous coefficient; μ is the growth rate; am is the cell yield; t is the retention time; N_H is the ammoniacal nitrogen; N_N is the nitrate nitrogen; and NKT is the Kjeldahl total nitrogen (ammoniacal and organic nitrogen).

When the system operates in steady state, the extracted sludge allows the average retention time of the sludge in the system (also called "sludge age" and denoted SRT) to be defined as the total mass of sludge divided by the quantity of sludge extracted daily.

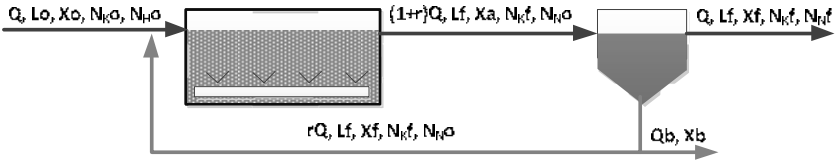


Figure 1.9. *Inlet and outlet characteristics of an activated sludge plant*

The balance sheet for organic matter is written:

$$V\,dL = Q\,Lo\,dt + rQ\,Lf\,dt - (1+r)\,Q\,Lf\,dt - (V\,\mu\,Xv\,dt)/am$$

In steady state, dL/dt = 0.

By setting the hydraulic retention time, t = V/Q, it becomes:

$$\frac{Lo}{t} + \frac{rLf}{t} - \frac{Lf}{t} - \frac{rLf}{t} - \frac{\mu Xa}{am} = 0$$

After simplification, we obtain:

$$\frac{Lo - Lf}{t} = \frac{Q(Lo - Lf)}{V} = \frac{\mu Xa}{am}$$

Hence:

$$\mu = \frac{am(Lo - Lf)}{Xa\,t} = \frac{\frac{amLo(Lo - Lf)}{lo}}{Xa\,t}$$

Consider the parameters mass charge (Cm) and carbon removal efficiency (ρ) defined by the relationships:

$$Cm = Lo/Xt$$

This is the daily BOD applied flux relative to the amount of active biomass present in the aeration tank.

ρ = (Lo-Lf)/Lo represents the purification efficiency.

The biomass growth rate is then expressed by the equation:

$$\mu = am\ Cm.\rho$$

Furthermore, the age of the G (or SRT) sludge is written as:

$$G = \frac{V\ Xa}{(1-\sigma)Q\ Xf + \sigma\ QXb} = \frac{Xa\ t}{(1-\sigma)Xf + \sigma Xb}$$

Assuming biomass losses (Xf) in the outlet water are negligible, the sludge age equation simplifies to:

$$G = \frac{Xa\ t}{\sigma Xb}$$

where rX is the rate of biomass growth. The biomass balance is written as:

$$rX = (\mu - b)V\ Xa = (1-\sigma)Q\ Xf + \sigma\ QXb = VXa/G$$

$$(\mu - b) = 1/G = am.Cm.\rho$$

1.4.7.1. *Empirical relations*

Based on the results obtained from real-world installations, wastewater treatment plant designers have determined the influence of temperature on the maximum autotrophic growth rate (Martin 1979):

$$\mu\ \text{autotrophs, maxi} = 0.13 * 1.09^{(T-15)}\ (\text{expressed in } d^{-1})$$

Assuming that the ammoniacal nitrogen concentration in the aeration tank can be estimated using the average concentration at the plant outlet, the minimum retention time required to maintain nitrification is expressed by the following equation:

$$G\ mini = 1/(\mu aut - baut)$$

$$G\ mini = \frac{1}{\mu aut - baut} = \frac{1}{\mu\ aut, mini\ \frac{(N-NH4)f}{(N-NH4)f + 1.5} - baut}$$

with:

– μaut: autotrophic growth rate;

– baut: endogenous respiration coefficient of autotrophs.

Neglecting baut, the equation finally becomes:

$$\text{G mini} = \frac{(\text{N} - \text{NH4})\text{f} + 1.5}{(\text{N} - \text{NH4})\text{f}} \frac{1}{0.13\ 1.09^{(\text{T}-15)}}$$

Another approach, for nitrification, involves defining a fictitious sludge age based solely on the sludge mass in aerated areas:

G aerated = X aerobic/sludge produced

the minimum value per G aerated is then generally taken as a function of the temperature:

G aerated mini = 4.5 x 0.914(T – 20)

The denitrification objective is achieved provided that a mass of MVS is maintained in the anoxic zone, such as: denitrified nitrogen/Xv anoxic < 2–4 $mg.g^{-1}$ $MVS.h^{-1}$.

The total mass of MVS to be maintained is then:

Xt = X aerobic + X anoxic

No excess sludge is produced for nitrogen removal.

1.5. Denitrification in activated sludge and denitrification rate

The implementation of nitrification in activated sludge depends on several factors that influence the sizing of the biological reactors.

The kinetics of the denitrification reaction with activated sludge can be expressed by:

$v_{DN} = dN\text{-}NO_3/dt = KX$

with:

– $dN\text{-}NO_3/dt$ = denitrification rate (g $N\text{-}NO_3.L^{-1}.h^{-1}$);

– $N\text{-}NO_3$ = nitrate concentration (mg $N\text{-}NO_3.L^{-1}$);

– t = time (hours);

– X = biomass concentration (mg $MVS.L^{-1}$);

– K = specific denitrification constant (mg $N\text{-}NO_3.mgMVS.h^{-1}$).

This expression indicates that the nitrate–time relationship is linear and independent of the nitrate concentration; that is, the rate is of zero order with respect to the nitrate concentration and is a function solely of the sludge concentration.

Since the denitrification reaction is controlled by active biomass, we write, for simplicity:

$$\Delta N\text{-}NO_3 = K\ Xa\ \Delta t$$

The denitrification rate is given by the following relationship, correlated with temperature according to Arrhenius law:

$$v_{DN}\ (T) = v_{DN}\ (20°C)\ \Theta^{(T-10)}$$

However, as the kinetics of denitrification can be disrupted by the presence of dissolved O_2, this inhibition is taken into account. The formula becomes:

$$vDN = \frac{1-1.42\ Y}{2.86}\ \frac{K\ S\ X}{Ks+S}\ \frac{N-NO3}{Kn+N-NO3}\ \frac{K'O}{K'O+O2}\ \eta$$

with:

– v_{DN}: denitrification rate ($mgN\text{-}NO_3^-.L^{-1}\ h^{-1}$);

– Y: cell synthesis efficiency of heterotrophs (mg MVS.mg^{-1} substrate consumed);

– K: maximum specific substrate consumption rate (h^{-1});

– X: biomass concentration in the reactor (mg MVS L^{-1});

– S: concentration of the biodegradable soluble substrate (mg L^{-1});

– KS: constant half-rate of substrate consumption reaction (mg L^{-1});

– $N\text{-}NO_3$: nitric nitrogen concentration ($mgN\text{-}NO_3^-.h^{-1}$);

– KN: half-rate constant of nitrate reaction ($mgN\text{-}NO_3^-.h^{-1}$);

– K′O: constant relative to the inhibition of O_2 for the reduction of nitrates ($mgO_2.^{-1}$);

– η: fraction of heterotrophic bacteria using nitrates as an O_2 source.

v_{DN} at 20°C is the denitrification rate at 20°C in the DN, generally equal to 3 mg $N\text{-}NO_3$ $gMVS^{-1}.h^{-1}$.

In activated sludge tanks, the vDN value often reported is 2.9–3.0 $gN\text{-}NO_3^-.h^{-1}$ $kgMVS^{-1}$ (Raboni 2014). Manufacturers use various empirical or semi-empirical relationships involving the specific rate of denitrification:

$$V\ specific\ DN_{20°C} = 0.029 + 0.03\ Cm$$

$$V\ specific\ DN\ (g\ N\text{-}NO_3.g\ MVS^{-1}.d^{-1}) = Q\ N\text{-}NO_3/V.X$$

Sludge production in anoxic-aerobic systems does not differ significantly from that of a comparable aerobic system, provided that the anoxic volume fraction is not too large.

K is a constant of the denitrification rate.

In an anoxic tank, the specific rate of denitrification represents the rate of nitrate reduction relative to the biomass concentration.

We can write:

$\Delta N\text{-}NO_3$ eliminated = Vanox vs Xa

where $N\text{-}NO_3$ is expressed in g $N\text{-}NO_3.h^{-1}$; Vanox is the volume of the anoxic tank (m^3); vs is the specific rate of denitrification in g $N\text{-}NO_3.SS^{-1}.h^{-1}$; and Xa is the concentration of suspended sludge (SS) in the tank ($g.m^{-3}$).

The observed values are highly variable across the different sites where they are measured. It is proposed that vs can be determined from the relationship:

vs = 0.03 Cm + 0.029

where Cm (kg $BOD_5.kg^{-1}$ SS^{-1}. d^{-1}) = Q Lo/Vanox Xanox

where Lo is the BOD of the influent ($mg.L^{-1}$) and Xanox is the sludge concentration in the anoxic tank ($mg.L^{-1}$).

We can then deduce:

vs = 0.03 Cm + 0.029

The various parameters related to denitrification kinetics are shown in Table 1.8.

Parameter	Units	Values
Cellular yield am	g $MVS.g^{-1}$ COD	0.40
Mortality coefficient b	g $MVS.g^{-1}$ TSS d^{-1}	0.07–0.10
Maximum specific growth rate µm	g $MVS.g^{-1}$ MVS. d^{-1}	3.2
Half-speed Ks	$g.m^{-3}$	9.0
Biomass COD	g $COD.g^{-1}$ MVS	1.42
Denitrifying bacteria fraction η	g $MVS.g^{-1}$ MVS	0.50

Table 1.8. *Parameters related to denitrification kinetics*

The relationship of vs with temperature is:

$$vs_{(T)} = vs_{20}\,\Theta^{T-20}$$

Θ often has a value of 1.026.

In a denitrification configuration with an upstream anoxic tank, sludge and nitrified effluent recycling is integrated.

For a recirculation rate (R) of 2:

vs adjusted = vso – 0.0166 Ln Cm – 0.0078

where vso is the specific velocity for an internal ratio (IR) equal to 1.

For a ratio of 3–4, the relationship becomes:

vs adjusted = vso – 0.029 Ln Cm – 0.012

Adjusted Cm is the mass load applied to the anoxic tank ($g.g^{-1}.\ d^{-1}$).

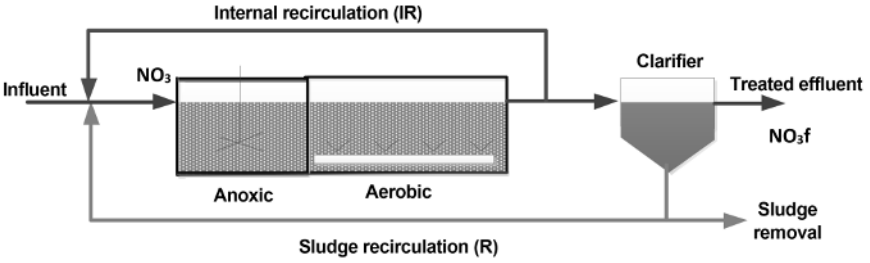


Figure 1.10. *Denitrification diagram with an anoxic tank installed upstream*

The active biomass in the anoxic tank is:

$$X\,\text{anox} = \frac{GQ}{V}\,\frac{am(Lo - Le)}{1 + b\,G}$$

In the case of alternating aeration in biological tanks, as with the Biodenitro™ process (see Figure 1.11), the specific rate vs is given by:

vs denitrification = 0.1754 An/(am G)

where An is the coefficient related to the consumption of O_2 (gO_2.g COD^{-1} removed).

The number 0.1754 is based on 2.86 g O_2 per g NO_3 and the assumption that only 50% of heterotrophic biomass is available for denitrification.

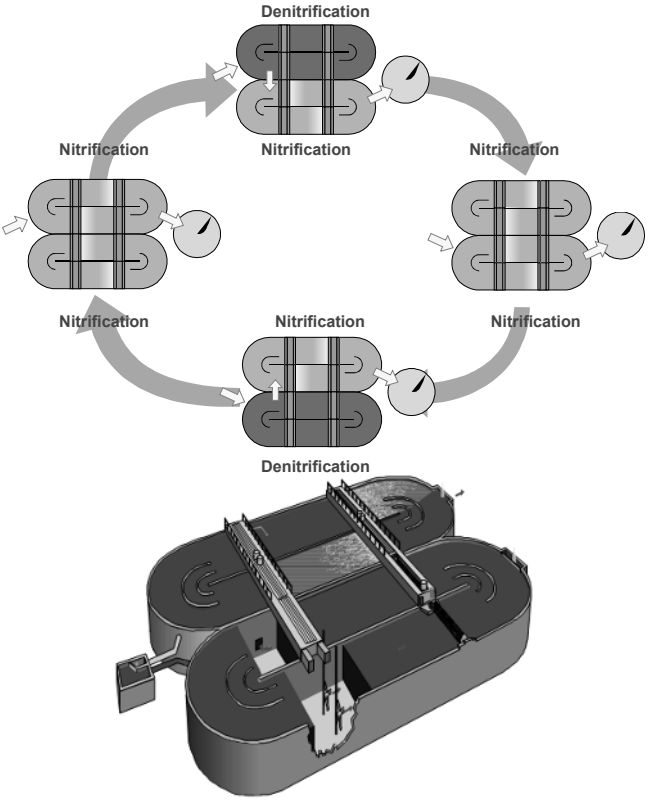


Figure 1.11. *Alternating aeration in oxidation channels: Biodenitro™ process (Veolia)*

Reducing nitrogen levels is important when effluents are discharged into sensitive water bodies that are subject to eutrophication.

1.5.1. *Specific anoxic zone for denitrification*

The total tank volume is estimated based on the desired volumetric load. This volume must then be distributed between the aerobic and anoxic zones.

Criteria:

– Mass charge in the anoxic tank Cm = 20 to 50 g $N.kg^{-1}$ $MVS.d^{-1}$.

– Volume of the anoxic tank V_{anoxic} = (25 to 40%) $V_{aerated+anoxic}$.

The volume of the anoxic tank varies depending on the nitrate flow recirculated from the clarifier and the flow supplied by the influencing material. The lower this flow, the smaller the tank.

1.5.2. *Estimation of excess sludge production*

The volume of the biological reactor (aerobic + anoxic) is defined based on the sludge age and the required sludge concentration (Xa). The Xa concentration is determined according to the treatment process and allows for the sizing of the clarifier, taking into account the Mohlman index and the most demanding hydraulic regime.

The total mass of sludge, combined with the degradation kinetics of carbonaceous and nitrogenous pollution, determines the tank volume based on the untreated water flow and the SS concentrations at the outlet. The clarifier's performance depends on various parameters: sludge mass flow, sludge thickening capacity, maximum sludge retention time and the structure's geometry.

The parameters influencing the production of biological sludge are the ratio TSS/ BOD_5, the mass load and the MVS/TSS ratio in untreated wastewater.

Sludge production can be obtained using the following relationship:

$$Xb\ excess = \Delta X = Xmin + Xdur + (0.83 + 0.2 \log Cm)\ BOD_5 + K' nitrified - Xs$$

with:

– Xdur: non-biodegradable fraction of MVS = 0.15 to 0.3 (MVS) untreated water;

– Xmin: mineral fraction of TSS = TSS x (100% of MV in TSS)/100;

– Cm: applied mass load kg $BOD_5.kg^{-1}MVS.\ d^{-1}$;

– BOD_5: concentration BOD_5 in the supply water;

– $K' = 0.17$ kg $nitrifying.kg^{-1}$ N nitrified;

– Xs: SS concentration in the outlet effluent ($mg.L^{-1}$);

– ΔX: excess sludge production requiring daily extraction (kg. d^{-1}).

In the case of biological phosphorus removal, the production of physicochemical sludge is taken into account.

1.5.3. *Carbon addition*

Denitrification is a carbon-consuming biological reaction. The denitrification capacity will therefore depend on the level of assimilable COD in the water being treated.

The requirement for assimilable COD for denitrification is approximately 4.6 kg $COD.kg^{-1}$ N, where N is the mass of $N.NO_3$ to be denitrified. This figure incorporates the needs for catabolism and anabolism. If the water to be treated does not contain enough assimilable carbon pollution, COD will need to be injected. The COD source that usually offers the best ratio of sludge quality produced to price is methanol.

Characteristics of methanol:

– Density = 0.79 $kg.L^{-1}$.

– COD = 1.5 $g.g^{-1}$ of methanol.

– BOD = 1 $g.g^{-1}$ of methanol.

– COD/N applied = 4–7.

– Kinetics = 2–3.5 g $N.NO_3.kg^{-1}$ MV/h.

– Sludge produced = 0.14 kg $TSS.kg^{-1}$ of methanol.

Methanol requires a specific biomass; maximum kinetics can only be reached after one month of injection.

The amount of carbon to inject is estimated by analyzing the remaining $N.NO_3$ in the treated water ($N.NO_{3outlet}$). We then calculate the flow of $N.NO_3$ that still needs to be treated by methanol injection:

– Flow $N.NO_{3\ \text{to be treated}} = (N.NO_{3inlet} - N.NO_{3desired\ outlet})Q$;

– Q = water flow rate to be treated in $m^3.d^{-1}$;

– COD flow to be injected ($kg.d^{-1}$) = flow $N.NO_{3\ \text{to be treated}} \times 4.6$;

– Methanol flow to be injected (kg/d^{-1}) = COD flow to inject/1.5;

– Methanol injection rate ($l.d^{-1}$) = methanol flow/0.79.

Carbon injection must take place in or just upstream of the anoxic tank (where denitrification occurs), in a well-agitated area. The flow rate of the carbon substrate is controlled by the flow rate of the water to be treated; it must be injected in slight excess. Carbon injection must be carried out at a constant volume (flow rate and duration) with sequential operation of the supply pumps. The pumps must be activated by the passage of a fixed volume of water to be treated. The pumps must operate efficiently to meet the reagent requirements for this volume of water, assuming a constant nitrate concentration. Regulation based on nitrate concentration measurements is possible.

1.6. Nitrification and denitrification processes in activated sludge

Activated sludge processes can be classified into two categories depending on whether the denitrification zone is installed upstream or downstream of the aeration zone.

1.6.1. *Pre-denitrification*

Wastewater and activated sludge come into contact and mix in an anoxic zone located upstream of an aeration zone. Nitrates formed in the aerobic zone by nitrification are recycled to the pre-DN. This process allows the use of the available organic carbon source in untreated wastewater. The denitrification yield is affected by the organic carbon concentration (BOD, COD) as well as other parameters such as biomass concentration (MVS) and temperature.

Since nitrification occurs after denitrification, the nitrates resulting from the oxidation of NH_4 in the aeration tank are ready to be discharged with the treated effluent if recycling does not occur.

Such a process typically produces an effluent with high concentrations of total nitrogen (NGL) below 20 mg N. L^{-1}.

1.6.2. *Post-denitrification*

This configuration includes an aerobic biological tank at the head of the line followed by an anoxic (non-mechanically aerated) tank. Self-generated endogenous organic matter and/or external carbon sources are used as carbon sources, because organic carbon (COD, BOD) is consumed during the first stage, with an energy cost

associated with aeration. Post-denitrification can lead to the release of ammonium in the anoxic zone if only self-generated endogenous organic matter is used as carbon.

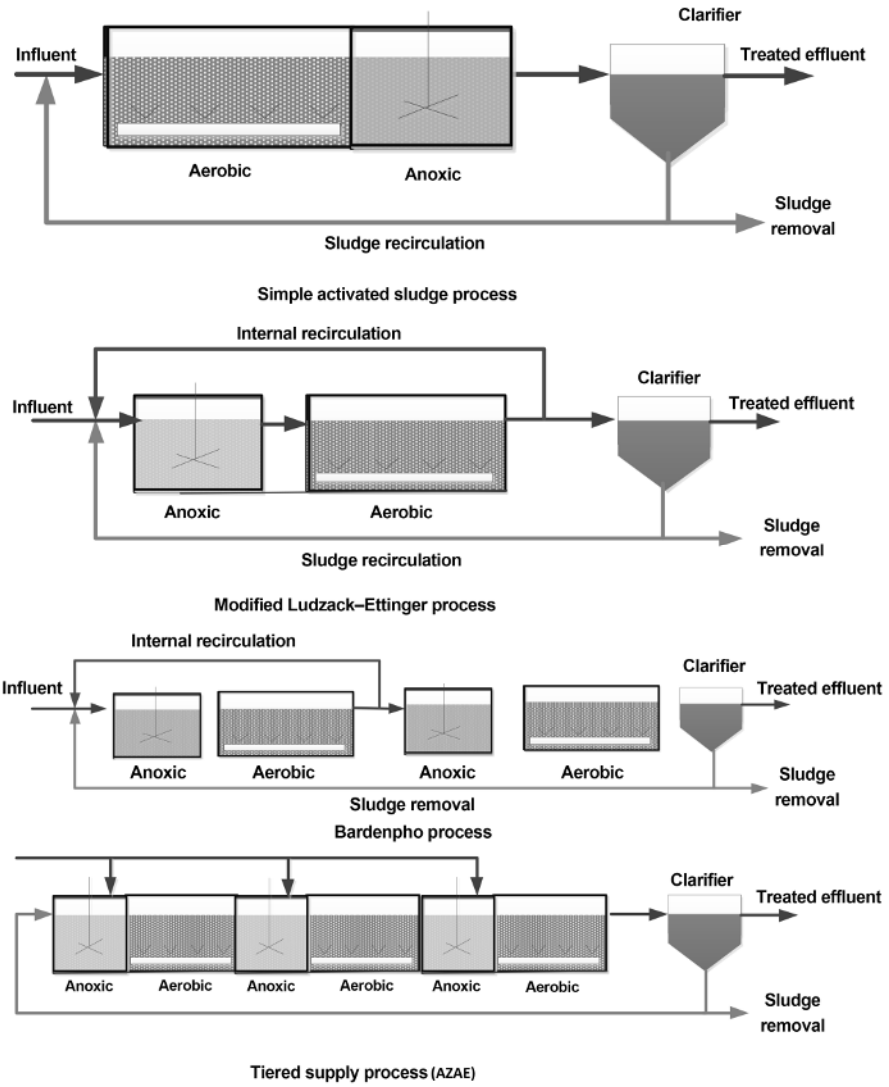


Figure 1.12A. *Some activated sludge nitrification–denitrification processes*

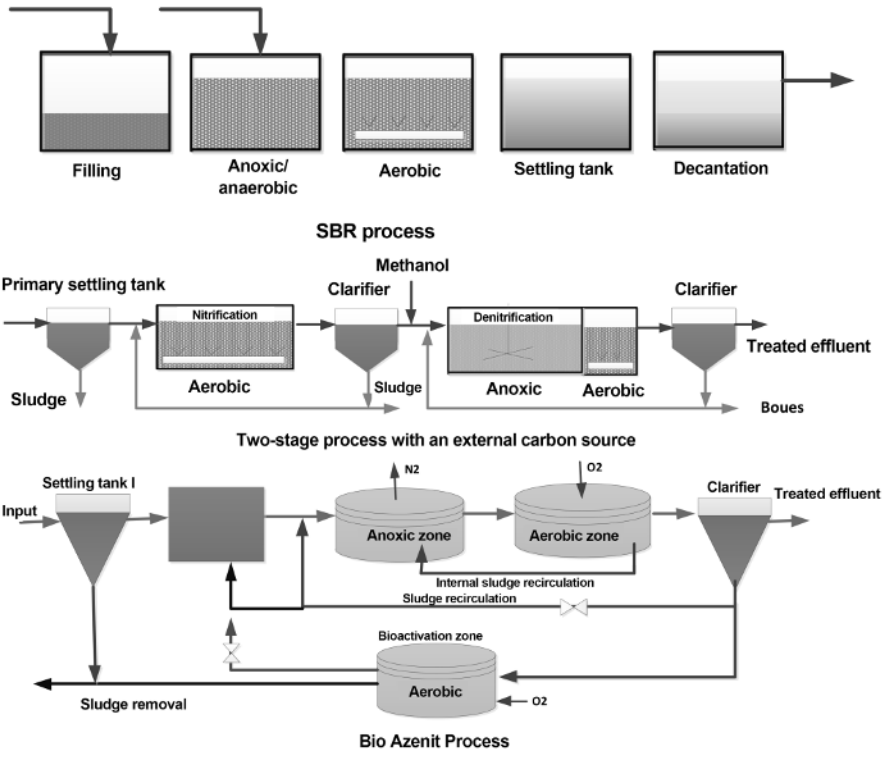


Figure 1.12B. *Some activated sludge nitrification–denitrification processes (continued)*

In practice, modernizing treatment plants involves adding a post-denitrification system followed by a final aeration stage to reoxygenate the effluent and refine the treatment process NH_4 (< 10 mg NGL.L^{-1}).

The main variants of the nitrification and denitrification processes combined in one or more reactors include:

– pre-denitrification (removal of nitrogen along with carbon from untreated wastewater);

– post-denitrification (elimination of nitrogen with carbon from endogenous respiration);

– alternating modes of aeration and anoxia in several stages;

– oxidation channels;

– batch sequencing reactors.

There are numerous denitrification processes. Figure 1.12 shows some of the processes proposed by manufacturers.

1.7. Autotrophic denitrification of activated sludge

The size of sulfur particles is an important parameter for sulfur–bacteria transfer. The smaller the size, the better the autotrophic denitrification process because the transfers are faster. The typical stoichiometric reaction of autotrophic denitrification with elemental sulfur involving *Thiobacillus denitrificans* is given in the following equation:

$$NO_3^- + 1.1\ S° + 0.4\ CO_2 + 0.76\ H_2 + 0.08\ NH_4^+ \rightarrow 0.5\ N_2 + 1.1\ SO_4^{2-} + 1.28\ H^+ + 0.08\ C_5H_7O_2N$$

The process of autotrophic denitrification with elemental sulfur can be presented as two consecutive reactions:

$$5S° + 6\ NO_3^- + 6H_2O \rightarrow 3\ N_2 + 5\ SO_4^{2-} + 4H_3O^+$$

$$11\ S° + 10\ NO_3^- + 4.1\ HCO_3^- + 0.5\ CO_2 + 1.72\ NH_4^+ + 2.54\ H_2 \rightarrow 0.92\ C_5H_7O_2N + 11\ SO_4^{2-} + 5.4\ N_2 + 9.62\ H^+$$

It can be noted that 2.51 g S° are consumed by g N-NO_3^-. Also, 7.54 mg.L^{-1} sulfates are produced per 1 mg N-NO_3.L^{-1} consumed (Gaid 1982). Different ratios (mg of sulfate produced per mg of denitrified N-NO_3^-) have been identified in the literature; namely, 1.5 (Liu 2009), 3.4 (Zhang 1999), 9.9 (Hashimoto 1987) and 11.1 (Schippers 1987).

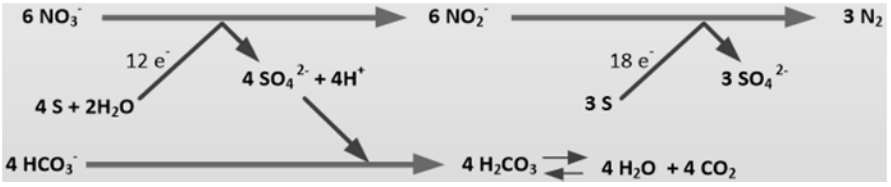
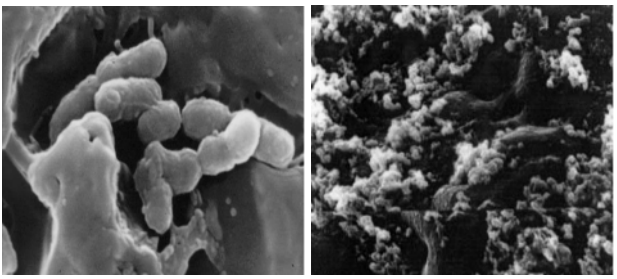


Figure 1.13. *Nitrate s-sulfur interactions and derivatives (Koenig and Liu 2001)*

Figure 1.13 summarizes the various chemical interactions occurring during autotrophic denitrification based on sulfur and limestone. According to this figure, *Thiobacillus denitrificans* acquire energy by oxidizing sulfur. Once the sulfur is oxidized, the released electrons will be used to reduce nitrates to nitrites, then to nitrogen gas.



(Blais 1994) (Moon 2004)

Figure 1.14. *Sulfur particles colonized by bacterial strains containing Thiobacillus denitrificans*

1.7.1. *Autotrophic denitrification mechanism with sulfur*

Denitrification using sulfur compounds as electron donors is an alternative to heterotrophic denitrification for wastewater with high nitrate concentrations and low organic matter content. This process is carried out by autotrophic denitrifiers such as *Thiobacillus denitrificans* and *Thiomicrospira denitrificans*. The energy required by these microorganisms comes from redox reactions with elements such as hydrogen or various reduced sulfur compounds acting as electron donors. Autotrophic denitrifiers use inorganic carbon compounds, for example, as a carbon source for growth. Consequently, compared to conventional heterotrophic denitrification, autotrophic denitrification has two clear advantages:

– no need for an external organic carbon source, which reduces the cost of the process;

– lower biomass production, which minimizes sludge handling (Claus and Kutzner 1985; Zhang and Lampe 1999).

Current values for maximum growth rates of autotrophic denitrifying microorganisms (0.11 to 0.20 h^{-1} (Claus and Kutzner 1985; Oh 2000)) are similar to heterotrophic values (0.062 to 0.108 h^{-1} (Wiesmann 1994)), whereas biomass yields are lower for autotrophic denitrifying microorganisms (0.40 to 0.57 g of $VSS.g^{-1}$ (Claus and Kutzner 1985; Oh 2000)) compared to denitrifying heterotrophs (0.8 to 1.2 g of $VSS.g^{-1}$ (Wiesmann 1994)).

Under ambient conditions, S° exists as a ring formed of eight sulfur atoms (S8), forming a crystalline solid. Many S8 allotropes exist, but the most common is α-S8, which is essentially insoluble in water. Given its low solubility, it is unlikely that the

microbial growth rates observed with S° can be obtained. It is thought that certain oxidizing bacteria produce extracellular enzymes that convert α-S8 into soluble polysulfides, Sx^{2-}, which can diffuse into a biofilm. Nanocrystalline S8 could also be an intermediate formed from Sx^{2-}. Other S°-oxidizing bacteria are known to have membrane-bound enzymes that obtain electrons by direct contact with the surface of S°, incorporating them directly into the cell.

The electron transfer mechanism is important because it can impact biofilm development and its kinetic behavior. If electron transfer is limited to bacteria in direct contact with S°, increasing biofilm thickness will not increase denitrification fluxes (the rate at which So removes NO_3^- per unit area of S°).

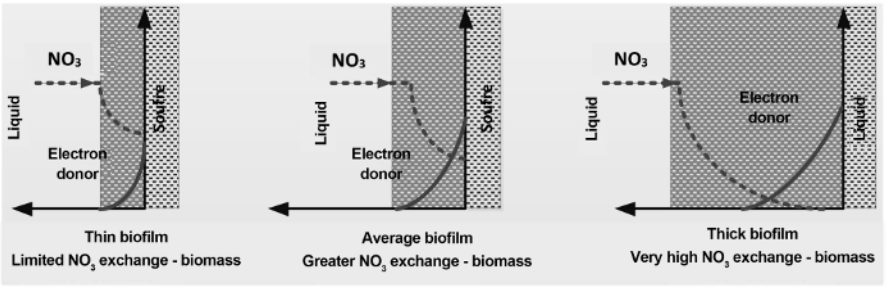


Figure 1.15. *Sulfur denitrification depending on biofilm thickness*

However, if soluble sulfur species diffuse into the biofilm, the increased biofilm thickness would provide more active biomass. This would allow for larger NO_3^- fluxes. The biofilm developing on S° is subject to two opposing diffusions: the donor and acceptor diffuse into the biofilms from opposite zones (see Figure 1.15). The kinetic behavior of counter-diffusion biofilms differs from that of conventional co-diffusion biofilms. Nitrate fluxes increase as biofilm thickness increases but eventually decrease when the film becomes too thick.

The decline is due to limited diffusion, because the donor and acceptor enter the biofilm from opposite sides and therefore have longer diffusion distances and thus lower concentrations when they meet (Essila 2000; Martin and Nerenberg 2012).

However, the form of sulfur (e.g. α-S8, polysulfides, S8 nanocrystalline) and the identity of the true electron donor are not fully explained. It is also unclear whether the soluble sulfur is only available to bacteria on the surface of the solid or whether it diffuses into the biofilm. This uncertainty and the complexity of counter-diffusion explain why the denitrification kinetics based on S° remain poorly characterized compared to other electron donors.

Given that the mechanisms of electron transfer from S° are not well established, the best approach might be to characterize denitrification rates as a function of biofilm development and NO_3^- concentration.

As a biofilm process, the S^o-based denitrification rate should be related to the surface area of the S^o rather than the S^o concentration (Sierra-Alvarez et al. 2007). This rate should vary depending on both the NO_3^- concentration and biofilm thickness.

1.7.2. *Autotrophic denitrification in free culture (activated sludge)*

Factors affecting the performance of autotrophic denitrification are those that directly impact microbial growth and activity, such as pH, temperature, dissolved oxygen, the presence of organic or inorganic contaminants and byproducts (nitrites, sulfates). Other parameters, such as sulfur and limestone particle size, the sulfur/limestone ratio, the nitrate concentration of the influencing material, the treatment flow rate and the hydraulic retention time, are also interrelated.

1.7.2.1. *Temperature*

The optimal temperature for the growth of denitrifying bacteria is approximately 30°C. Amatya et al. (2005) studied the effect of temperature on biological denitrification and established that the denitrification rate is significant above 16°C and maximal at 30°C, with a nitrate removal efficiency of 76.9% in columnar reactors. Temperature not only plays an important role in bacterial cell growth but also has a direct effect on gene expression of enzymes involved in denitrification. Maximum denitrification was reached at temperatures ranging between 33 and 35°C (Oh et al. 2000). However, another study showed that it was possible to completely denitrify all nitrates at 10°C, except that the duration of the treatment is significantly longer compared with the other temperatures mentioned previously. Trouve et al. (1998) isolated cryophilic strains of *Thiobacillus denitrificans* capable of denitrifying at temperatures as low as 5°C with denitrification efficiency similar to that occurring at 20°C. The problem with low temperatures lies in the fact that the denitrification process would require longer incubation periods. Complete nitrate removal at 10°C requires 36 to 78 hours, and the same strains may require approximately 48 to over 120 hours of incubation using a batch process.

Furthermore, depending on the sulfur source used, it appears that at a temperature of 5°C and a pH between 6 and 8, nitrate removal is more efficient in the presence of thiosulfate compared to elemental sulfur. A classification describing the affinity of *Thiobacillus denitrificans* regarding the available sulfur source has been proposed: $S_2O_3^{2-} > FeS > FeS_2 > S°$ (Ben Khaled 2016).

1.7.2.2. *pH*

Autotrophic denitrification with sulfur leads to acidification of the environment, caused by a decrease in pH and alkalinity. The reduction of one mole of nitrate produces 1.10 moles of H^+ and leads to bicarbonate consumption. The pH parameter is important for the growth and survival of any microorganism, and since autotrophic denitrification is regulated by microorganisms, pH plays a critical role in the NO_3-S° system. The optimal pH for most autotrophic denitrifying bacteria ranges between 6 and 9 (Holt et al. 1994). However, a study by Koenig also demonstrated that autotrophic denitrifying bacteria such as *Thiobacillus denitrificans* have an optimal pH in the range of 6.8 to 8.2 and that a pH below 6 is detrimental to the process (Koenig and Liu 2002). At low pH (below 6), nitrogen oxide reductases are inhibited, particularly those reducing nitrous oxide (N_2O) and the overall denitrification rate decreases due to the accumulation of N_2O (Bergaust et al. 2010). In order to achieve complete denitrification, the pH of the system must be neutralized with suitable substrates such as sodium bicarbonate.

1.7.2.3. *Dissolved oxygen*

Dissolved oxygen (DO) poses a major problem in denitrification mechanisms, whether with heterotrophic or autotrophic bacteria. Since it is part of the basic nutritional requirements of microorganisms, oxygen is preferentially assimilated compared to nitrates present in the effluents to be treated. Furthermore, the electrochemical potential of oxygen is higher than that of nitrates. Consequently, oxygen competes with nitrates as the final electron acceptor and inhibits denitrification.

1.7.2.4. *Influence of the S/N ratio*

The effect of different S/N ratios on the efficiency of autotrophic sulfur denitrification was studied by Campos et al. (2008). They varied the concentrations of thiosulfate as a sulfur source relative to that of nitrates. When the S/N ratio increased from 1.16 to 2.44 g $S\text{-}S_2O_3.g^{-1}N\text{-}NO_3$, an accumulation of nitrites was observed, and denitrification could not be completed. However, with a higher S/N ratio (6.67 $g.g^{-1}$), nitrates were completely reduced to nitrogen gas at the start of the experiments, but nitrites still accumulated afterwards (Ben Khaled 2016).

1.7.2.5. *Influence of the S°/X ratio*

The specific rate of denitrification is given by:

$$\mathrm{vDN} = \frac{1}{\mathrm{X}} \frac{\partial N}{\partial t}$$

where X is the concentration of biomass in the reactor ($mg.L^{-1}$). This relationship shows that v_{DN} is proportional to the S°/X ratio when this ratio is less than 50 $g.g^{-1}$. Under constant operating conditions (pH between 7.5 and 8; alkalinity between 270 and 280 mg $CaCO_3$; temperature = 20°C), the denitrification kinetics show the evolution of the various elements (nitrates, pH, sulfates), as represented by a few examples (see Figure 1.16). These results allow us to calculate the denitrification rates v_{DN} (mg $N\text{-}NO_3^-.g.MVS^{-1}.h^{-1}$) depending on the S°/X ratio.

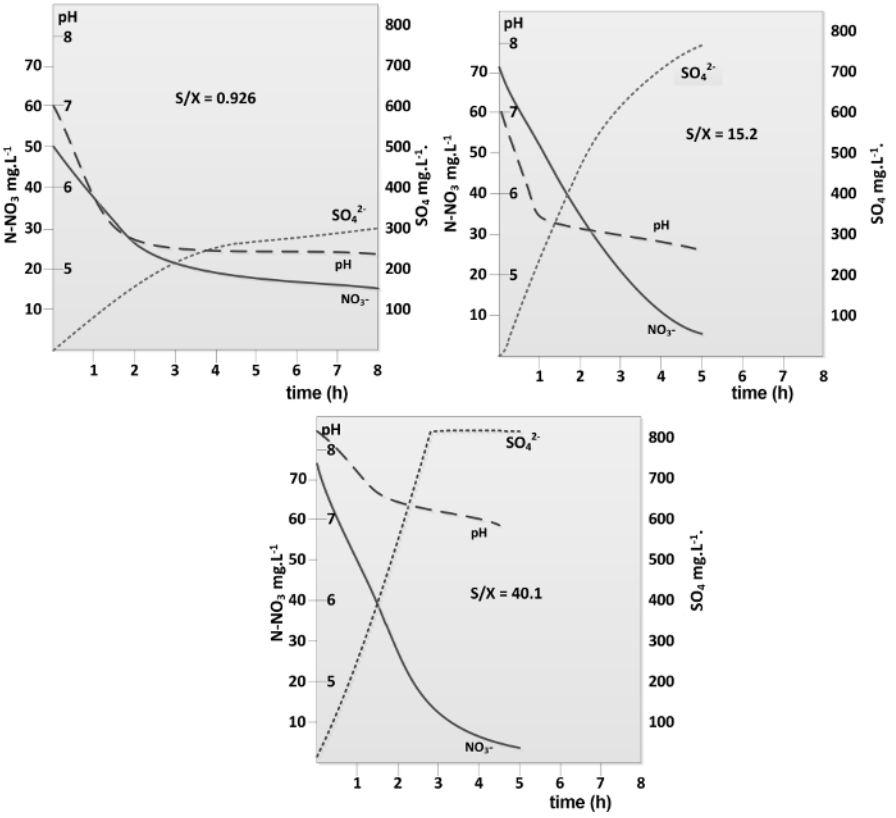


Figure 1.16. *Influence of S°/X ratio on sulfur denitrification*

We obtain the following:

$$vDN = \frac{5\ S°/X}{9.1 + S°/X}$$

The rate of nitrate–sulfur denitrification follows a Monod-type law. It depends on the ratio of sulfur to sludge present at the initial moment of the reaction.

1.7.2.6. *Influence of alkalinity and alkalinity ratio*

According to the sulfur denitrification reaction, autotrophic denitrifying bacteria require 3.91 g of alkalinity in the form of $CaCO_3$ per gram of reduced N-NO_3 to control the pH:

$$55\ S + 44\ CaCO_3 + 50\ NO_3^- + 18\ H_2O + 4\ NH_4^+ \rightarrow 4\ C_5H_7O_2N + 25\ N_2 + 55\ SO_4^{2-} + 44\ Ca^{2+} + 24\ HCO_3^-$$

The denitrification rate (mg N-NO_3.g $MVS^{-1}.h^{-1}$) obeys the following relationship, for an alkalinity between 200 and 340 mg $CacO_3.L^{-1}$:

$$vDN = \frac{12.5\ [\text{alkalinity}]}{37 + [\text{alkalinity}]}$$

1.7.2.7. *Influence of temperature*

The denitrification rate is in the form:

$$v_{DN} = ks\ \Theta^{(T-10)}$$

In the presence of sulfur, the relationship is as follows:

$$v_{DN} = 2.01 \times 1.12\ \Theta^{(T-10)}$$

1.7.3. *Autotrophic denitrification in a biofilter*

1.7.3.1. *Submerged biofilter*

The Veolia process involves a denitrification bioreactor filled with a granular mixture of elemental sulfur and maerl (Gaid 1982; Ratel and Debrieu 2003). Upon exiting the columns, the water passes through post-treatment systems for filtration, reoxygenation and disinfection. This is done to avoid excessive prolonging of the seeding period of the columns with the autotrophic denitrifying bacteria *Thiobacillus denitrificans*. Due to the low growth rate, the system is enriched with phosphates, nitrates, ammonia and bicarbonates by recirculating the enrichment solution for two weeks. To prevent pressure losses due to increased biomass, backwashing with air is performed every 10 days. The process has some drawbacks related to the significant amount of sulfates produced and the need for low flow rates to optimize denitrification efficiency.

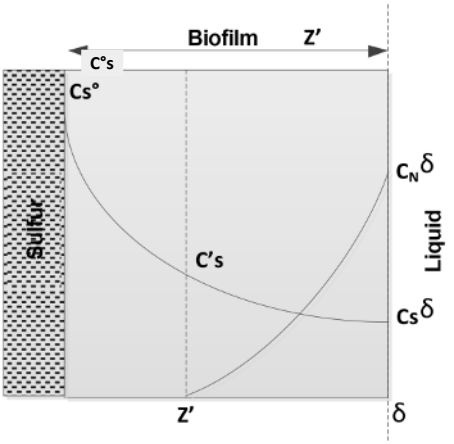


Figure 1.17. *Evolution of the various elements in a sulfur–nitrates–biomass system*

Figure 1.17 shows the evolution of sulfur and nitrates in a biofilm developed with sulfur. Cs and C_N represent the concentrations of S and N-NO_3 in the biofilm, respectively. Z is the distance in the film from the surface S. δ is the thickness of the biofilm. Sulfur (C°s) dissolves in the biofilm at a constant rate. This concentration decreases by C°s at Cs during passage through the biofilm. Due to its insolubility in water, sulfur does not penetrate the water. Nitrates C_N^{δ} are reduced by passage through the biofilm to a zero concentration at the point Z = Z′. Assuming homogeneity of the biofilm, establishing a mass balance leads to the following denitrification equation. For a low ratio S/X:

$$vDN = \frac{ks}{Y} C°s \left(\frac{Ds}{ks}\right)^{1/2} \frac{\rho b}{\rho S} \left(\frac{A}{V}\right) \frac{Cs}{X}$$

with:

– ks: first-order rate constant in intrafilm reactions (d^{-1});

– C°s: sulfur concentration at the start of the biofilm ($M.L^{-3}$);

– Ds: sulfur diffusion coefficient ($L^2.t^{-1}$);

– ρb: sludge density ($M.L^{-3}$);

– ρs: sulfur density ($M.L^{-3}$);

– A: biofilm area (L^2);

– Cs: sulfur concentration in the reactor ($M.L^{-3}$);

– X: biomass concentration in the reactor ($M.L^{-3}$);

– Y: stoichiometric coefficient equal to the ratio of the rate of sulfur consumption to the denitrification rate;

– A/V: ratio of the surface area to the volume of granular sulfur ($L^2.L^{-3}$).

For a high S/X ratio, the denitrification rate becomes independent of sulfur and is written as:

$$vDN = \frac{ks}{Y} C°s \frac{\rho b}{\rho S}$$

The Monod saturation constant (Ks) is low (Claus and Kutzner 1985; Batchelor 1987). The reaction that takes place within the biofilm is zero order. Because the substrate penetrates the biofilm pores slowly, the zero-order reaction within the biofilm becomes a half-order reaction at the biofilm surface (Koenig and Liu 2001).

The flow passing through the biofilter is considered a piston flow. We can write:

$$\frac{\partial C}{\partial \delta} = -v'DN \frac{A}{Q}$$

where C is the concentration, δ is the thickness of the biofilm, Q is the flow rate, A is the surface area traversed by the flow and v′DN is the denitrification rate per unit volume of the biofilter.

Since the reaction in the biofilm is half-order:

$$v'DN = k\, C^{1/2}$$

Replacing v′DN, it becomes:

$$-\frac{\partial c}{\partial t} = -k\sqrt{C}$$

where k is the half-order reaction constant per unit volume of biofilter; i.e.:

$$\sqrt{Ct} = \sqrt{Co} - \frac{kt}{2}$$

1.7.3.2. *Influence of volumetric load*

The influence of volumetric load (kg $N\text{-}NO_3.m^{-3}$ sulfur. d^{-1}) on the denitrification yield (R) is given by the relation:

$$R\ (\%) = a\ Cv^{b}$$

where a and b are constants related to R and the percentage of sulfur contained in the biofilter.

Table 1.9 summarizes the values of a and b.

Sulfur (%)	R (%)
5	$R = 0.29\ Cv^{-1.45}$
15	$R = 0.70\ Cv^{-1.40}$
30	$R = 0.90\ Cv^{-1.13}$
45	$R = 1.05\ Cv^{-0.82}$
100	$R = 1.23\ Cv^{-0.68}$

Table 1.9. *Denitrification efficiency as a function of sulfur load*

1.7.3.3. *Influence of alkalinity*

The influence of alkalinity on the denitrification rate (mg $N\text{-}NO_3$ removed.h^{-1}) is indicated by the following:

$$\mathrm{vDN} = 125\ \frac{\text{alkalinity}}{41.6 + \text{alkalinity}}$$

Alkalinity is expressed in °f:

$$1°f = 10\ \text{mg}\ CaCO_3.L^{-1}$$

1.7.3.4. *Influence of the sulfur/limestone ratio*

Alkalinity is an important parameter that regulates denitrification kinetics. Carbonate consumption linked to the formation of H^+ ions tends to rapidly lower the pH and limit bacterial activity.

Because the biological reaction of autotrophic denitrification with sulfur generates H^+ ions, the process consumes alkalinity and therefore requires a buffer substrate to prevent the pH from dropping. Limestone is used as the solid phase to neutralize the acidity generated during the autotrophic denitrification reaction with

sulfur (Batchelor 1978; Gaid 1982). Batchelor (1978) and Gaid (1982) showed that limestone is a well-suited neutralizing agent for autotrophic denitrification with sulfur and that the ratio of initial alkalinity to the theoretically required alkalinity in wastewater must be at least 0.5 for effective nitrate removal. The sulfur-to-limestone ratio is generally determined by the alkalinity of the tributary. Alkalinity is the ability of water to maintain a stable pH (buffering capacity) and is reflected by the presence of hydroxide ions (OH^-), bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}). Therefore, consuming bicarbonate leads to a decrease in alkalinity.

Theoretically, during an autotrophic denitrification process, 1 mg of denitrified N-NO_3 leads to the consumption of 4.5 mg of $CaCO_3$ (Sengupta et al. 2007). However, other authors have reported relatively different results, indicating that 1 mg of denitrified N-NO_3 would consume 5.6 mg of $CaCO_3$ (Zhang and Lampe 1999) and 4.77 mg of $CaCO_3$ (Moon 2006). During autotrophic denitrification, the dissolution of limestone to neutralize acidity results in the transformation of bicarbonate into carbonic acid in the presence of H^+ ions, thus generating the release of carbon dioxide.

The neutralization step proceeds according to the following equations:

$$CaCO_3(s) + H^+ \rightarrow HCO_3^- + Ca^{2+}$$

$$HCO_3^- + H^+ \rightarrow H_2CO_3$$

$$H_2CO_3 \rightarrow H_2O + CO_2$$

A 1:1 S:C ratio ensures a nitrate removal efficiency of approximately 98% (Gaid 1982). A 1:1 ratio would be optimal to achieve a high nitrate removal efficiency with low sulfate production (Sikora 1976; Schippers 1987). Certain factors such as affluent pH, the initial alkalinity and the limestone requirement can influence the optimal S:C ratio via denitrification (Liu 2002). Indeed, if the initial alkalinity is present in the form of bicarbonate and the pH of the affluent to be treated is around 8, an S:C ratio greater than 2:1 is sufficient to ensure effective denitrification. This requirement depends largely on the minimum pH allowed for maintaining denitrification, which determines the S:C ratio. Therefore, the optimal S:C ratio would be defined as the proportion at which the volumetric loading is optimal.

1.7.3.4.1. Sulfur and limestone configuration in the bioreactor

The lining configuration of the denitrifying biofilter varies depending on the process applied and the type of wastewater to be treated. The biofilter lining can have several configurations in which granular elemental sulfur and granular limestone are mixed. One original idea is to use mixtures of sulfur with calcium carbonate. This solution is simple to implement with marine maerl, which consists

of 80 to 85% calcium carbonate, 10 to 15% magnesium carbonate, 0.65% calcium phosphate, 0.60% sulfur and 1% organic nitrogen (Gaid 1982).

The applied volumetric loading rates are higher due to the continuous pH neutralization by the maerl (see Table 1.10 and Figure 1.18). The total filtration height is 1.75 m.

	Composition of the biofilter		
	Sulfur 50% Maerl 50%	**Sulfur 45% Gravel 55%**	**Sulfur 100%**
Material dimensions (mm)	Sulfur: 2–5 Maerl: 2–5	Sulfur: 2–5 Gravel: 3–7	Sulfur: 2–5
Volumetric load (kg N-NO_3 applied.m^3. d^1)	3	1	1.85

Table 1.10. *Biofilter performance with different material compositions*

The majority of studies cited in the literature refer to a DASC where sulfur and limestone are mixed in the same bioreactor, generally referred to as a "fixed-bed reactor" (Gaid 1982; Zhang 2004; Sengupta et al. 2007; Zhou et al. 2011).

The autotrophic denitrification kinetics with sulfur follow a half-order reaction and imply that the denitrification efficiency is dependent on the specific surface area of the reactive sulfur particle (Koenig and Liu 2001).

The autotrophic denitrification process can use electron donors such as S°, sulfide (S^{2-}), SCN^-, thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), metallic elements and ions. The biomass yield of autotrophic denitrifiers is generally lower than that of heterotrophic denitrifiers, which is advantageous due to reduced sludge management.

However, metallic elements and ions, as well as sulfur and its compounds, generate harmful chemicals (Di Capua et al. 2019). S^{2-} can exist in water in the form of molecular hydrogen sulfide (H_2S) or hydrogen sulfide ions (HS^-)/sulfide (S^{2-}) according to the medium conditions (Wiemann et al. 1998; Di Capua et al. 2019). S^{2-} presents serious environmental challenges due to its toxicity, corrosivity and odor (Di Capua et al. 2019). Furthermore, it is an inhibitor for many microorganisms when present in high concentrations, including autotrophic denitrifying bacteria (Cardoso et al. 2006).

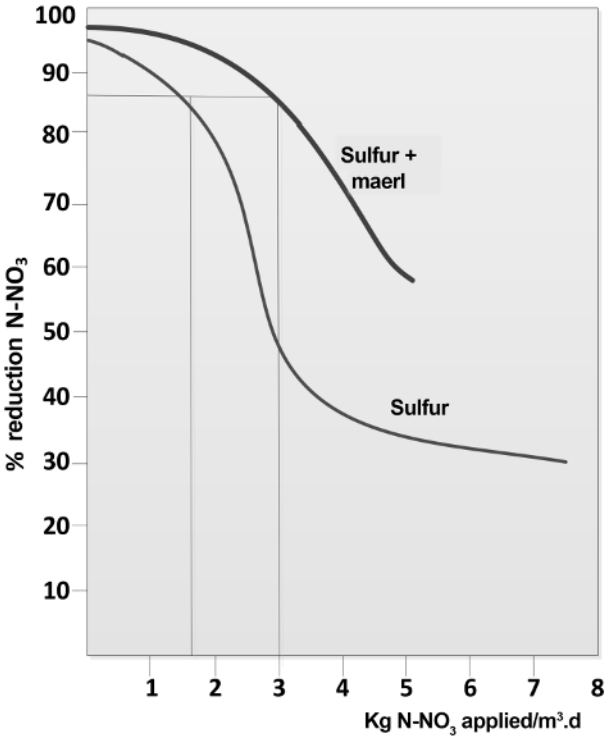


Figure 1.18. *Denitrification efficiency as a function of the applied load for two sulfur loads*

The two-step process is the preferred method for autotrophic denitrifiers using S, probably due to lower electronic requirements compared to direct oxidation of S^{2-} into SO_4^{2-} (Moraes et al. 2012). The formation of S as an intermediate product could lead to the formation of a white/yellowish color that would disappear when the S is subsequently consumed. Furthermore, the consumption of S leads to a fluctuation in alkalinity since the oxidation of S into SO_4^{2-} leads to a decrease in carbonates and a drop in pH.

1.8. Nitrogen treatment by anammox bacteria

The oldest and still most widespread biological process for removing nitrogenous species from wastewater is nitrification–denitrification, that is, the nitrification of ammonia into nitrate, followed by an organic reduction of nitrate into diatomic nitrogen. For sustainability reasons, particularly due to the energy required to supply

oxygen and the amount of organic matter needed, which could otherwise be used to produce methane fuel, other potential nitrogen removal processes have emerged. Among these, biological processes have been implemented, using a unique group of bacteria called "anammox," which anaerobically convert ammonia into nitrogen gas while using nitrite as an electron acceptor. Thus, the ammonia is oxidized solely to nitrite and then denitrified. This reduces both the oxygen and organic matter requirements for denitrification.

In this process, only about half of the ammonia is initially oxidized to nitrite by conventional ammonia-oxidizing bacteria. Anammox bacteria then use this nitrite as an electron acceptor under anoxic conditions to oxidize the remaining half of the ammonia, converting both into nitrogen gas.

Classically, ammonia is oxidized to nitrite by ammonia-oxidizing bacteria (AOB), and the biochemical process is carried out by the *Nitrosomonas* and *Nitrospira*. Next, nitrites are oxidized to nitrates by nitrite-oxidizing bacteria (NOB) with the help of microorganisms such as *Nitrobacter* and *Nitrospira*. The denitrification process is primarily carried out by heterotrophic bacteria, such as *Paracoccus denitrificans* and *Pseudomonas stutzeri*, which require organic carbon resources as electron donors to reduce nitrogen oxides to nitrogen gas.

Due to the oxygen supply required for the nitrification process and the organic matter requirements for the denitrification process, the conventional activated sludge process is an energy-intensive process.

New bacterial communities have been discovered, which show advantages over conventional nitrifying or denitrifying bacteria, such as higher affinity for the substrate, better physicochemical tolerances and/or lower greenhouse gas emissions. Overall, these new microbial communities could offer new possibilities for efficient and energy-saving nitrogen removal from wastewater. These microbial communities are capable of using other electron acceptors or donors, and of offering substantial advantages over the classical nitrogen removal process, such as lower production of N_2O, energy savings during aeration and organic carbon savings during anoxic denitrification. In addition, they can better cope with adverse environments such as low O_2 or ammonia deficiency conditions.

The anammox (anaerobic ammonium oxidation) process is a new pathway in the nitrogen cycle (see Figure 1.19). This process involves oxidizing ammonium under anaerobic conditions using nitrite as an electron acceptor instead of oxygen. It results in the final production of N_2 (inert gaseous product) and nitrate. It is carried out by autotrophic microorganisms not yet cultured in pure strains but for which enrichment conditions are known in laboratory reactors. The anaerobic oxidation of ammonium (anammox) is a new metabolic pathway (Lam et al. 2009) in the nitrogen cycle,

offering new opportunities for process engineers and microbiologists. The existence of these bacteria capable of carrying out the anaerobic oxidation of ammonium (anammox), with nitrates and/or nitrites, to nitrogen gas opens up new perspectives because they bring simplifications to the treatment processes.

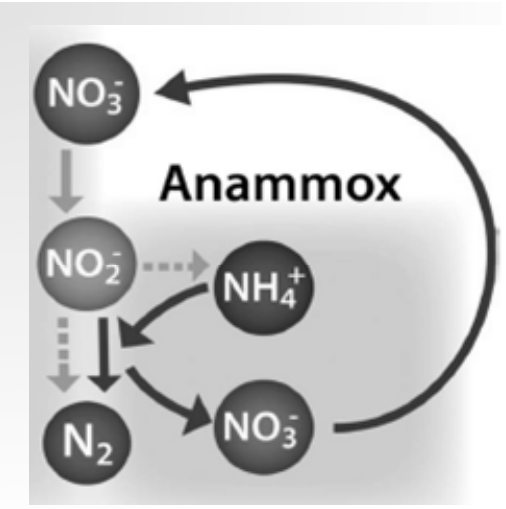


Figure 1.19. *Simplified diagram of the desammonification and denitrification process*

1.8.1. *Anammox characteristics*

The anammox process is currently linked to a monophyletic group of bacteria belonging to the phylum of *Planctomycetes*. The bacteria that carry out this process are still poorly understood, but are quite unique.

1.8.2. *Microbiological characteristics*

Anammox bacteria are cells approximately 1 μm in diameter with a cell envelope organization very different from that of Gram-negative or Gram-positive bacteria. They consist of a cell wall lacking peptidoglycan, a cytoplasmic membrane and an intracytoplasmic membrane. Their cytoplasm is divided into three compartments: the paryphoplasm, the riboplasm and the anammoxosome (Niftrik et al. 2004). This last compartment is specific to anammox bacteria.

They contain a specific intracellular compartment, the anammoxosome, which occupies a significant volume of the cell and where the anammox process takes

place. The membrane of this vesicle is made up of lipids with a particular chemical structure, ladderanes, consisting of three or five fused cyclobutane rings. These molecules with constrained geometry form an extremely dense barrier, thus greatly reducing the membrane's permeability to small molecules, particularly hydrazine (N_2H_4), an intermediate and highly toxic product of the anammox reaction (Kuenen 2008).

Currently, five genera of anammox bacteria have been (provisionally) defined: *Brocadia*, *Kuenenia*, *Anammoxoglobus* and *Jettenia* regarding freshwater species, as well as Scalindua for marine species.

In summary, anammox bacteria are characterized by several remarkable properties:

– They all possess an anammoxosome, a large membrane-bound compartment within the cytoplasm where anammox catabolism takes place.

– The lipids constituting the membranes of these bacteria contain a high proportion of ladderane-type fatty acids, such as pentacycloanammoxic acid, which are currently unique in biology.

– Hydrazine N_2H_4 and hydroxylamine NH_2OH, toxic to most living beings, are metabolic intermediates in the anammox process.

– Their growth rate is extremely slow, with a doubling rate of nearly two weeks. These microorganisms are strict anaerobes and chemolithoautotrophs (Niftrik 2004). Their doubling time is approximately 1.8 to 11 days (Magrí 2012), their cell yield is very low (0.11 g $VSS.g^{-1}$ $N\text{-}NH_4^+$) and they are very sensitive to variations in environmental conditions, which makes them extremely difficult to cultivate (Strous et al. 1999; Ni and Meng 2011).

Methods for obtaining pure cultures of Anammox bacteria are complex and require very strict protocols. However, enrichment cultures are available, and everything we know about them derives from these enrichments (Dalsgaard et al. 2005).

1.8.3. *Biochemistry of anaerobic oxidation of ammonium*

The biochemistry of anammox bacteria is not yet fully understood. It is accepted that the anammox process takes place in the anammoxosome and that its objectives are the synthesis of adenosine triphosphate (ATP) and carbon fixation (Niftrik et al. 2004). According to Figure 1.20, the proposed mechanism indicates that nitrites are reduced to hydroxylamine (NH_2OH) by a nitrite reductase (NIR). Ammonia and hydroxylamine are then used by hydrazine hydrolase to produce hydrazine (N_2H_4).

Hydrazine oxidoreductase oxidizes hydrazine into N_2 and releases four protons and four electrons. The four released electrons are used with five protons from the riboplasm by nitrite reductase (Nir) to complete the catalytic cycle. All of these reactions establish a proton gradient, which is used for ATP synthesis catalyzed by adenosine triphosphatase (ATPases) bound to the anammoxosome membrane. The synthesized ATP is then released into the riboplasm (Jetten et al. 1999; Schalk 2000; Jetten et al. 2001; Brandes et al. 2007).

The reactions are as follows:

$$NO_2^- + 5H^+ + 4es \rightarrow NH_2OH + H_2O$$

$$NH_2OH + NH_3 \rightarrow N_2H_4 + H_2O$$

$$N_2H_4 \rightarrow N_2 + 4H^+ + 4es$$

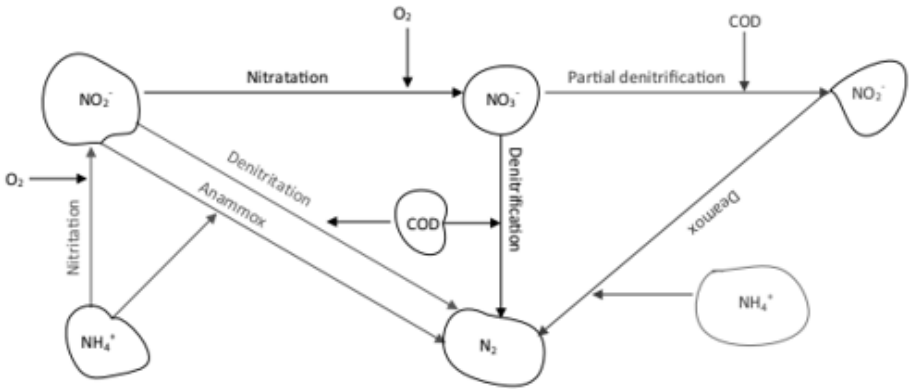


Figure 1.20. *Overall diagram of anammox involvement (Kosgey et al. 2022)*

Excess nitrite (0.3 mol/mol ammonium) is oxidized anaerobically to nitrates. The electrons from this oxidation are probably used for CO_2 fixation (Van de Graaf et al. 1995).

The overall assessment of reactions, proposed by Strous et al. (1999), is presented here:

$$NH_4^+ + NO_2^- = N_2 + 2H_2O \text{ (x 15)}$$

$$CO_2 + 2\ NO_2^- + H_2O = CH_2OH + 2\ NO_3^-$$

$NH_4^+ + 1.32\ NO_2^- + 0066HCO_3^- \rightarrow 1.02N_2 + 0.256\ NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$

The catabolic reaction is replicated 15 times to bind a molecule of carbon dioxide with nitrites. These, by releasing electrons, lead to the anaerobic production of nitrate.

1.8.4. *Factors influencing the growth of anammox bacteria*

To date, five genera of anammox bacteria have been identified in the literature, forming the monophyletic group Brocadiales (Ali et al. 2013). These different genera appear to be able to occupy different ecological niches. The metabolism of anammox bacteria allows them to colonize habitats and adapt to new situations by using a variety of electron donors and acceptors (e.g. manganese, iron oxides and formate) (Strous et al. 2006). Indeed, these bacteria regularly compete with other bacteria in the nitrogen cycle (nitrifying and denitrifying bacteria) and with archaea due to biological interactions between these microbial groups for nitrogen and the CO_2.

A potential for specific niche differentiation has thus been observed for each genus of Anammox in natural and anthropogenic systems, and even dominance in certain environments (such as oxygen-deprived areas). The most obvious examples include marine environments, wastewater treatment plants, freshwater, estuaries and deep-sea hydrothermal vents (Hassan 2018).

These observations have led to numerous hypotheses regarding the environmental conditions conducive to the enrichment of anammox bacteria.

1.8.5. *Salinity*

Brummer suggests that the species of *Planctomycetes* in freshwater environments differ from those found in marine environments (Brummer et al. 2004). In a number of estuaries, the anammox level decreases with increasing salinity (Trimmer et al. 2003). The type of anammox communities is changing, favoring the growth of genera best adapted to increased salinity (Kartal et al. 2006; Hassan 2018).

1.8.6. *Presence of organic matter and/or nutrients*

A change in environmental conditions (e.g. for the start-up of a bioreactor for anammox enrichment) can also trigger a change in the dominant anammox genus (Van Der Star et al. 2007).

Nitrite and micronutrient concentrations, the use of alternative energy sources, the presence of carbon sources (propionate or acetate) (Kartal et al. 2007, 2008) as well as anthropogenic inputs of nutrients can lead to marked changes in the structure of the community (Li et al. 2011; Hassan 2018).

1.8.7. Candidatus Brocadia *and* Kuenenia

These species have attracted considerable attention because they are the genera commonly found in wastewater treatment plants. The niche difference between these two genera is still unknown. Thus, the change in dominance over time of *Brocadia* towards *Kuenenia* was observed in a membrane biofilm reactor (MBR) (Van Der star et al. 2008) and in batch reactors. In both cases, the affinity for nitrite was assumed to be the limiting factor.

Based on these observations, the authors hypothesized that anammox bacteria have very low growth rates, and therefore, long startup periods are necessary for their enrichment. Periods of 58 days (Liao et al. 2007) and 60 days (Dapena-Mora et al. 2007) have been reported in batch bioreactors (SBRs) using methanogenic granular sludge or activated sludge as the inoculum.

Table 1.11 summarizes the growth and functioning parameters of various anammox bacteria.

In large-scale installations, generation times of 11 days (Van Der Star et al. 2007) and 27 days (Joss et al. 2009) were measured. These characteristics make the implementation of anammox bacteria in a treatment process challenging in terms of achieving enrichment and then maintaining activity in the process.

Strategies have been reported in the literature aimed at reducing startup time and/or promoting biofilm formation (Fernández et al. 2008; Hassan 2018), including the use of membrane bioreactors (Van Der Star et al. 2008).

However, exposure to inhibitory conditions could lead to complete reactor failure, regardless of the strategy used to conserve biomass and maintain anammox bacteria. Therefore, restoring full wastewater treatment capacity would be a very lengthy process. Very little information is available on the sensitivity of Anammox bacteria to compounds commonly found in wastewater. Substrate concentration, oxygen levels, temperature, pH, etc., must be considered for the application and industrialization of the process (Hassan 2018).

Parameters	*Brocadia anammoxidans*	*Brocadia sinica*	*Kuenenia stuttgartiensis*	*Scalindua* sp.	*Scalindua profunda*	*Jettenia caeni*
Growth temperature (°C)	20–43	25–45	25–37	10–30	15–45	20–42.5
Growth pH	6.7–8.3	7.0–8.8	6.5–9.0	6.0–8.5	7.4	6.5–8.5
Max growth rate (μmax, d^{-1})	0.065	0.098	0.062–0.084	0.048	–	0.048
Doubling rate (d)	10.7	7	8–11	–	–	–
Cell yield am (mmol C mmol N^{-1})	0.07	0.063	–	0.03	–	0.056
km per NH_4 (μM) (5*m°)	< 5	28 +/- 4	–	3	–	17.1 +/- 4.3
km per NO_2 (μM)	< 5	34 +/- 21	0.2–3	0.45	–	35.6 +/- 0.92

Table 1.11. *Physiological and kinetic characteristics for anammox bacteria (Ni et al. 2016)*

1.8.8. *Oxygen*

Therefore, restoring full wastewater treatment capacity would be a very lengthy process. Very little information is available on the sensitivity of anammox bacteria to compounds commonly found in wastewater. Substrate concentration, oxygen levels, temperature, pH, etc., must be considered for the application and industrialization of the process (Jung et al. 2007). It has been reported that the anaerobic oxidation process of ammonium is reversibly inhibited by dissolved oxygen at a low level (< 1% of air saturation) (Strous et al. 1997). Conversely, irreversible inhibition occurs at high concentrations (> 18% of air saturation) (Egli et al. 2001). Therefore, dissolved oxygen must be strictly controlled in processes to prevent process shutdown and protect the maintenance of anammox bacteria.

1.8.9. *Temperature*

The maximum activity of anammox was observed between 35 and 40°C in a sequential batch reactor (Dosta et al. 2008). Egli et al. (2001) reported an optimal temperature of 37°C for anammox. Temperatures above this range cause an irreversible drop in anammox activity due to cell lysis, while temperatures below 15°C make the process unstable (Dosta et al. 2008).

In marine environments where *Candidatus scalindua* is the only type detected, optimal anammox activity was measured at 12°C (Rysgaard et al. 2004). These results suggest that anammox activity differs depending on the genus considered, as well as that an anammox process could be acclimatized and then used to treat wastewater at different temperatures.

1.8.10. *Substrate concentrations*

Anammox bacteria can be inhibited by their own substrates, nitrites and ammonium ions. Generally, they are more sensitive to high nitrite concentrations than to high ammonium ion concentrations. It is important to control the concentrations of these substrates in bioreactors and to adjust their stoichiometry in the flux, even though the literature does not offer a consensus regarding the limiting values (thresholds) of inhibition.

1.8.11. *Nitrite*

Numerous studies have confirmed that nitrite is a feedback inhibitor of anaerobic ammonium oxidation when it reaches excessively high concentrations. However, the

observed inhibition thresholds vary between 48 and 750 mg $N\text{-}NO_2.L^{-1}$ according to the operating procedures.

These threshold differences can be attributed to differences in biomass characteristics (anammox species) and/or operational conditions (pH, reactor configuration, temperature, substrate concentrations, etc.).

Anammox bacteria can be inhibited by their own substrates, nitrite and ammonium. Generally, they are more sensitive to high nitrite concentrations than to high ammonium concentrations. It is important to control the concentrations of these substrates in the bioreactors and to adjust their stoichiometry in the influencing system.

In an SBR containing aggregated anammox biomass (80% anammox bacteria/total bacteria), an immediate and complete inhibition of the anammox process was observed at nitrite concentrations above 100 mg $N\text{-}NO_2.L^{-1}$ (Strous et al. 1999).

On the other hand, by keeping the initial ammonium concentration constant at 42 $mg.L^{-1}$ in different batches, Egli et al. (2001) observed complete inactivation of the process at values above 185 mg $N\text{-}NO_2.L^{-1}$. Using granular biomass, improved nitrite tolerance was observed, with the IC_{50} at 350–400 mg $N\text{-}NO_2.L^{-1}$ (Dapena-Mora et al. 2007; Cho et al. 2010; Hassan 2018), probably due to the limitation of substrate diffusion within the granules.

Other authors have used gel supports on which anammox bacteria are trapped. Batch experiments have shown that when these supports are immersed in solutions with concentrations higher than 274 mg $N\text{-}NO_2.L^{-1}$, anammox activity decreases. Finally, a 90% drop in anammox activity was observed when the nitrite concentration reached 750 mg $N\text{-}NO_2.L^{-1}$ over a period of seven days in a bioreactor with continuous supply (Kimura et al. 2010).

1.8.12. *Ammonium ion (NH_4^+)*

In most wastewater treatment plants, the ammonium ion is one of the main forms of nitrogen. The literature reports that the anammox process is inhibited only at ammonium concentrations above 1,000 mg $N.L^{-1}$ (Strous et al. 1999), but it is accepted that it is NH_3 rather than NH_4^+ that is the real inhibitor. Similarly, in short-term trials (batch tests), the median NH_3 inhibitory concentration is 38 mg $N.L^{-1}$, whereas in long-term tests in sequential batch reactors, process instability is observed beyond 20–25 mg-$N.L^{-1}$ of ammonia (Dapena-Mora et al. 2007).

1.8.13. *Inhibition mechanisms and pH*

Ammonium does not easily diffuse across the lipid membrane of bacterial cells, which is not the case for ammonia (Kadam and Boone 1996). Thus, there is a chemical equilibrium between the molecule and the ion that can be affected by the pH (Mosquera-Corral et al. 2005):

$$NH_3/NH_4^+$$

$$NH_4^+ + OH^- \longleftrightarrow NH_3 + H_2O,$$

The diffusion of ammonia into the cell changes the intracellular pH and neutralizes the transmembrane potential, which can cause cell death (Martinelle and Westlund 1996).

Similarly, the inhibition of the anammox process by nitrite is not caused by the nitrite itself but by nitrous acid (Fernández et al. 2012), and pH can also affect the chemical balance:

$$HNO_2/NO_2^-$$

$$HNO_2 + H_2O \longleftrightarrow NO_2^- + H_3O^+$$

Thus, with a pKa of 9.24 and 3.3 for the acid–base pairs NH_3/NH_4^+ and HNO_2/NO_2^-, the pH of the anammox process is adjusted between 6.5 and 8.8, with an optimal pH of 8 (Oshiki et al. 2011). The pH must therefore be rigorously controlled during the operation of the process (Mosquera-Corral et al. 2005; Hassan 2018).

1.8.14. *Implementation using anammox bacteria*

Deammonification is a biological treatment process used to convert ammonia into nitrogen gas. It includes both nitritation and anaerobic ammonia oxidation processes (anammox).

Nitritation, the aerobic oxidation of ammonia N ($N\text{-}NH_4$) in nitrite-nitrogen ($N\text{-}NO_2^-$), is carried out by aerobic autotrophic bacteria that oxidize ammonia (AerAOB), a well-known process in wastewater treatment that constitutes the initial stage of biological nitrification of $N\text{-}NH_4$ into nitrates ($N\text{-}NO_3^-$).

These aerobic oxidizing bacteria are nitrifying bacteria, chemolithotrophic microorganisms, and include species of the genera *Nitrosomonas* and *Nitrosococcus*. They obtain their energy through the oxidation of nitrogenous compounds. More specifically, AOB obtain their energy by oxidizing ammonia/ammonium to nitrite.

AOB have a complex internal membrane system. Indeed, this is the location of key enzymes, such as ammonia monooxygenase, which oxidizes ammonia to hydroxylamine, or hydroxylamine oxidoreductase, which oxidizes hydroxylamine to nitric oxide. The latter is oxidized to nitrite by an unidentified enzyme (Kuypers et al. 2018). AOB produce nitrites according to the reaction shown below (Graham and Jolis 2017).

AOB and anammox bacteria can be combined to convert ammonia into nitrogen gas using a process called partial nitritation-anammox (PNA), where the AOB convert the available ammonia into nitrites. Then, anammox converts the NO_2^- and remaining $NH4^+$ into nitrogen gas (N_2), using NO_2^- as an electron acceptor:

$$NH_4^+ + 2.4\ O_2 + 6.72\ HCO_3^- \rightarrow 2.51\ NO_2^- + 1.04\ H_2O + 0.12\ C_5H_7NO_2 + 6.51\ H_2CO_3$$

In PNA, approximately 50% of the ammonium ions are initially converted to nitrites under aerobic conditions by AOB. In a second step, the remaining ammonium and nitrite ions are converted to nitrogen gas and a small fraction of nitrates by anammox bacteria (Graham and Jolis 2017):

$$2NH_4^+ + 0.34\ NO_2^- + 0.13\ H^+ + 1.38\ O_2 + 2.05\ HCO_3^- \rightarrow 1.02\ NO_2^- + 0.26\ NO_3^- + 3.09\ H_2O + 0.032\ C_5H_7NO_2 + 1.89\ H_2CO_3$$

The PNA process is commonly used in secondary wastewater treatment. In systems engineering, PNA can be found as a one- or two-stage application. The single-stage solution refers to carrying out the PNA process in a single reactor, while two-stage solutions maintain aerobic and anaerobic conditions in separate, but still connected, tanks.

The anammox reaction is carried out by anaerobic, autotrophic *Planctomycetales*, which are NH_4^+-oxidizing bacteria (AnAOB) that use NO_2^- as an electron acceptor to anaerobically oxidize the NH_4 into N_2 (Strous et al. 1999). Throughout the process, approximately 89% of the nitrogen is inorganic (NH_4 and NO_2) and ends up as N_2 gas and approximately 11% in the form of NO_3^-.

In the conventional denitrification process, NH_4^+ is first oxidized into NO_2^-, then into NO_3^- by autotrophic bacteria. Then, NO_3^- is biologically reduced into N_2 by heterotrophic bacteria (denitrification) with organic substrate consumption under anoxic conditions. Aerobic biological nitrification is well known and is carried out by the bacteria *Nitrosomonas* for the oxidation of NH_4^+ into NO_2^- and by *Nitrobacters* for the oxidation of NO_2^- into NO_3^-.

In the nitritation/denitritation process, the NH_4^+ is oxidized into NO_2^-, then biologically reduced to N_2 by heterotrophic bacteria consuming organic matter substrates under anoxic conditions (see Figure 1.21).

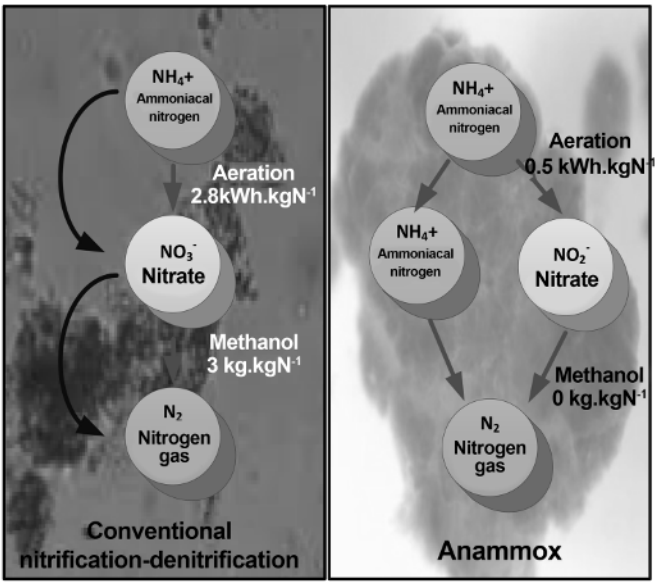


Figure 1.21. *Energy consumed by the conventional and anammox processes*

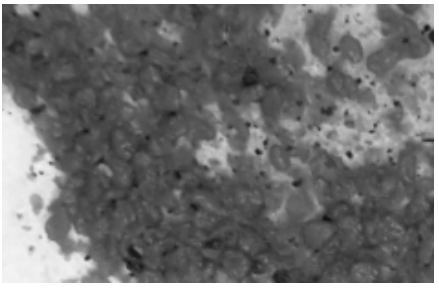


Figure 1.22. *Anammox bacteria in granules*

In the deammonification process, aerobic nitritation of NH_4^+ into NO_2^- represents the transformation of approximately 55% of the NH_4^+ of the influent. The remaining NH_4^+ is oxidized anaerobically with NO_2^- and into N_2 in the anammox process.

In practice, the process avoids the need for complete oxidation of NO_2^- and NO_3^-, followed by denitrification of NO_3^- into nitrogen gas.

Compared to conventional nitrification and denitrification, the demands for aeration and a carbon source are reduced by more than 50% and 100%, respectively.

It should be noted that biomass production is higher in the conventional process than in deammonification (20 g of COD biomass versus 3 g of COD biomass per mole of removed NH_4^+).

In the absence of dissolved oxygen, heterotrophic bacteria can oxidize the organic substrate with NO_2^- or NO_3^- as electron acceptors to reduce oxidized nitrogen to N_2 gas.

The reduction reactions of NO_2^- and NO_3^- are called denitritation and denitratation. The stoichiometries of biological denitrification and denitrification reactions with acetate consumption and heterotrophic biomass growth are as follows:

– Denitritation:

$$1.0.\ NO_2^- + 1.0\ H^+ + 0.24\ NH_4^+ + 0.975\ CH_3COO^- \rightarrow 0.5\ N_2 + 0.24\ C_5H_7O_2N + 0.015\ CO_2 + 0.735\ HCO_3^- + 1.235\ H_2O$$

– Denitratation:

$$1.0.\ NO_3^- + 1.0\ H^+ + 0.33\ NH_4^+ + 1.45\ CH_3COO^- \rightarrow 0.5\ N_2 + 0.33\ C_5H_7O_2N + 0.13\ CO_2 + 1.12\ HCO_3^- + 1.62\ H_2O$$

Therefore, 6.6 g of COD (acetate) is required per g of $N\text{-}NO_3$; for the denitritation of $N\text{-}NO_2$, just under 30%, that is, 4.5 g COD (acetate) per g de $N\text{-}NO_2$.

Anammox involves exoenergetic reactions for the oxidation of $N\text{-}NH_4$ into $N\text{-}NO_2$ and the absorption of CO_2 and nutrients through the growth of autotrophic anammox bacteria. The overall cellular synthesis reaction is written (Strous et al. 1999):

$$1.0.\ NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$$

During the anaerobic oxidation of ammonia, some nitrates are formed from nitrites, which can provide reducing power for the fixation of carbon dioxide (Schmidt et al. 2002). The removal of 1 mole of NH_4 requires 1.32 moles of NO_2 and produces 0.26 moles of NO_3.

The combination of nitritation and deammonification reactions leads to an overall reaction:

$$1.0.\ NH_4^+ + 0.804\ O_2 + 0.071\ HCO_3^- \rightarrow 0.436\ N_2 + 0.111\ NO_3^- + 0.009\ C_5H_7O_2N + 0.028\ CH_2O_{0.5}N_{0.15} + 1.038\ H^+ + 1.46\ H_2O$$

The stoichiometric ratio between nitrate production and ammonium consumption for anammox bacteria is 1:0.382. For field deammonification reactions, ammonia reductions of 90 to 95% and total nitrogen reductions of 80 to 85% (WERF 2014) have been obtained.

Table 1.12 summarizes the advantages of the deammonification process compared to the conventional denitrification process.

Parameters	Deammonification	Nitritation – denitritation	Nitrification – denitrification
O_2 demand	1.84 gO_2.gN-NH_4^{-1} removed	2.65 gO_2.gN-NH_4^{-1} removed	3.3 gO_2.gN-NH_4^{-1} removed
COD demand (acetate)	0	4.5 g acétate COD.gN-NO_2 removed	6.6 g acetate COD.gN-NO_2 removed
Biomass production	0.12 g MVS gN-NH_4^{-1} removed	1.5 g MVS gN-NH_4^{-1} removed	1.93 g MVS gN-NH_4^{-1} removed

Table 1.12. *Comparison of conventional deammonification and denitrification processes (WERF 2014)*

1.8.14.1. *Influence of the C/N ratio*

Theoretically, 2.86 g of COD would be needed to eliminate 1 g of N-NO_3^- (Hellinga et al. 1999; Daigger 2014). However, since a significant amount of COD would be oxidized during the aeration phase, the C/N ratios actually required for complete nitrogen removal are higher (5 to 7) (Hellinga et al. 1999; Sahinkaya et al. 2014). C/N ratios of 1.94 to 3 would be required for the removal of nitrogen via the NO_2^- pathway (nitritation–denitritation). This nitrogen removal option saves approximately 40% of COD and 25% of aeration costs compared to nitrification/denitrification since the NH_4^+ is only oxidized into NO_2^- as opposed to NO_3^- in the case of classic nitrification/denitrification (Hellinga et al. 1999; Van Kempen et al. 2001; Noutsopoulos et al. 2018; Dobbeleers et al. 2020).

Coupling nitritation with denitrification requires control of parameters such as pH, OD, temperature and the sludge retention time (SRT)/hydraulic retention time (HRT) ratio or even G/t (sludge age/HRT). This is done to suppress NOB growth while promoting AOB growth. Generally, biological reactors operate at a temperature of 30–40°C and a variable pH between 7 and 8.

1.8.14.2. *Influence of temperature*

Between 5 and 20°C, NO_2^- oxidizes more quickly than NH_4^+, leading to the generation of NO_3^-. However, at higher temperatures (30–40°C), the reverse is also true. For example, the difference in growth rates is the basis of the Sharon process, which seeks to limit nitration while promoting nitration. During the process, a combination of high temperatures (30–40°C) and the reaction time (1 day) leads to the leaching of NOB. Essentially, the high growth rates of AOB compensate for the biomass removed from the system.

1.8.14.3. *Inhibition of anammox bacteria reactions*

Furthermore, high temperatures and high concentrations of NH_4^+ and NO_2^- also limit NOB activity because they respectively dissociate into free ammonia (FA) and free nitrous acid (FNA), both of which are more toxic to NOBs than to AOBs (Anthonisen et al. 1976; Duan et al. 2021).

The inhibitory concentrations of FA and FNA for NOB are 0.10 to 1 $mg.L^{-1}$ and 0.011 to 0.070 $mg.L^{-1}$, while those for AOB are 10 to 150 $mg.L^{-1}$ and approximately 0.40, respectively (Anthonisen et al. 1976; Chen et al. 2020).

1.8.14.4. *Influence of pH*

To completely remove nitrogen from wastewater by nitritation–denitritation, a COD supplement is required for low-COD wastewater. The process could be implemented in a continuously stirred system in which a COD source is added during a non-aerated phase (Lemaire et al. 2008). Since aeration lowers the pH and denitrification raises it, this parameter is used to control the aeration and dosing of the COD sources. This strategy is beneficial because the pH is adjusted during nitritation and denitrification, thus eliminating the need to add an alkali (Hellinga et al. 1998). If the incoming wastewater contains high C/N ratios (> 2), then the same wastewater could be used as a COD source by implementing a phased feeding strategy, as Lemaire et al. (2003) demonstrated. Other process control methods include sequencing operations to define time intervals. Strategies based on the oxidation–reduction potential (ORP) could also be used to regulate the process, as previously demonstrated (Claros et al. 2012).

1.8.15. *Nitrogen removal processes using anammox*

Wastewater treatment plant outlet standards comply with European standards. These standards, based on the quality of the receiving environment, most often require nitrogen treatment in addition to organic matter (COD and BOD) and particulate matter.

Ammoniacal nitrogen (NH_4) is the main nitrogen compound contained in the treatment plant's inlet water and must be treated in situ.

With the digestion process and the combination of "thermal hydrolysis + digestion", the effluents generated during the digested sludge dewatering stage return to the start containing high concentrations of ammoniacal nitrogen but very little organic carbon (transformed into methane in the digester).

Although representing only 1 to 2% of the plant's inlet flow, these return flows can contain 15 to 30% of the nitrogen load entering the plant. Table 1.13 illustrates the impact of these return flows from the dewatering plant according to the treatment process.

Sludge treatment process	**Concentration N-NH_4^+ ($mg.L^{-1}$)**	**Nitrogen load recycled**
Digestion alone	500–1,500	≈ 10–15%
Digestion with thermal hydrolysis	2,000–4,000	≈ 25–30%

Table 1.13. *NH_4 concentrations for the returning loads from the digester*

This nitrogen overload results in a significant increase in the cost of biologically treating the water supply by generating:

– an additional energy cost linked to the increased oxygen requirements for processing nitrogen overloads during the nitrification phase;

– an additional cost for reagents related to the potential need for a carbon substrate (methanol) during the denitrification phase;

– a risk of nitrogen non-compliance when the size and variability of return loads are not correctly taken into account when setting the initial size of the water piping.

Thus, separate treatment of digested recirculated water upstream of the water line is paramount if we wish to avoid the consequences linked to implementing boosted digesters (external inputs, prior thermal hydrolysis of sludge, etc.).

Several technical solutions have been developed to implement the deammonification process. Table 1.14 presents the technical solutions, which differ in

the methods for growing and preserving anammox bacteria, the number of stages, the configuration and the control strategies. These include fluidized bed reactors, SBRs, moving bed reactors (MBBRs) and rotating biological contactors.

Process	Company
DEMON®	APG Neuros (USA)
Anita™ Mox	Veolia (France)
ANAMMOX®	Paques (the Netherlands)
DeAmmon	Purac/Läckeby AB (Sweden)
Terra-N	Clariant/SÜD-Chemie AG (Germany)
Sharon®	Université (the Netherlands)

Table 1.14. *Processes using anammox bacteria*

1.8.15.1. *DEMON® process*

The DEMON® process, acronym for "deammonification", involves two stages: the partial nitritation of ammonia and the subsequent anaerobic oxidation of residual ammonia by nitrites into nitrogen gas. Partial nitritation requires only 40% oxygen compared to conventional nitrification. No carbon (methanol) dosing is necessary due to the autotrophic nature of the process.

The DEMON® process is an SBR process with aerated/anaerobic conditions controlled by supply, aeration and mixing mini-cycles in non-aerated conditions that maintain aerobic/anaerobic conditions. The aeration periods promote the oxidation of some of the NH_4^+ into NO_2^-. Cycle times depend on the concentration of NH_4^+. When these concentrations are high, the cycle times are longer.

Controlling dissolved oxygen is important, and it must be maintained at values below 0.6 mg $O_2.L^{-1}$. Such control is important to avoid the inhibition of anammox due to nitrite accumulation, and also to ensure the survival of NOB.

The enormous impact on resource consumption demonstrates the nitrogen cycle. Denitrification and nitration do not occur; nitritation only partially occurs. Total nitrogen removal is achieved using only a very small amount of oxygen.

Figure 1.23. *DEMON® process*

1.8.15.2. *Anita™ Mox process*

A high ammonia concentration and a low chemical oxygen demand (COD) are characteristic of the water discharged from the dewatering of digested sludge. This ammonia load is generally returned to the main wastewater stream. However, its treatment by conventional nitrification/denitrification proves very costly or, in some cases, can disrupt downstream sludge digestion processes aimed at reducing sludge volume or improving biogas production.

Reducing sludge volumes while producing energy (conventional anaerobic digestion, thermal reduction processes) generates effluents. This recirculated water, heavily laden with NH_4^+, is often sent back to the start of the treatment line and consequently increases the nitrogen load of the water system by 15 to 20%.

This has a direct impact on the capacity of aeration systems, the size of biological tanks and the quality of effluents. These effluents can exceed the standards set for nitrogen without resorting to a post-denitrification step, which involves the costly injection of methanol as a carbon substrate.

To remedy this, AnoxKaldnes™ (Veolia) developed Anita™ Mox, an alternative to conventional nitrification/denitrification processes that limits the impact of digestion on the main treatment line of the wastewater treatment plant. Anita™ Mox is another application of a well-established technology, the mobile bed biofilm reactor (MBBR) or IFAS. It combines a single-stage MBBR with a proven aeration control strategy. Anita™ Mox significantly reduces the ammonia load (> 85–90%) and

eliminates 75 to 85% of the nitrogen in the main treatment process, making it an ideal solution for modernizing an existing, overloaded wastewater treatment plant at a competitive cost. An anammox process comprises two stages: aerobic nitritation by ammonia-oxidizing bacteria and anoxic oxidation of ammonia by anammox bacteria. In Anita™ Mox, both steps take place simultaneously in a single reactor and both aerobic and anoxic reactions occur in a single MBBR equipped with plastic supports specifically designed to retain biofilm. Specific pH, temperature and oxygen level conditions are maintained in the reactor; these provide ammonia-removing bacteria with an optimal environment, allowing them to develop as biofilms on the substrates and preventing their leaching.

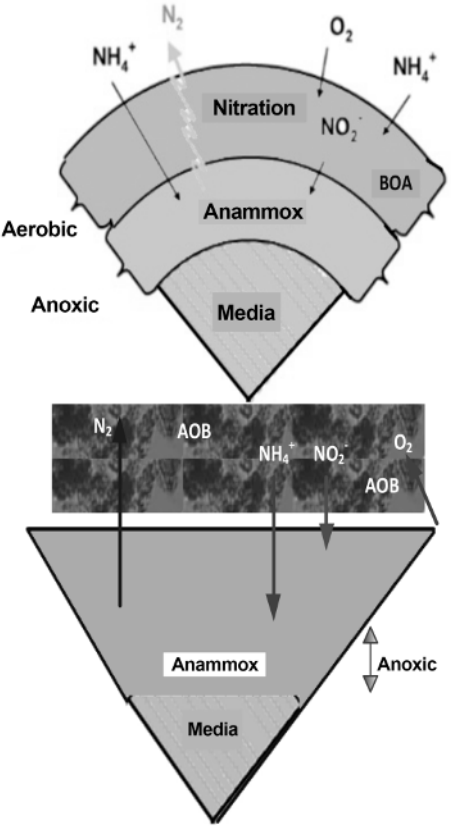


Figure 1.24. *Anita™ Mox principle: a) MBBR; b) IFAS*

On the substrate biofilm, aerobic and anaerobic zones develop simultaneously. AOB develop on the outer layer, exposed to the NH_4^+ concentration and dissolved oxygen. Anammox develops on the inner layer close to the substrate (see Figure 1.24).

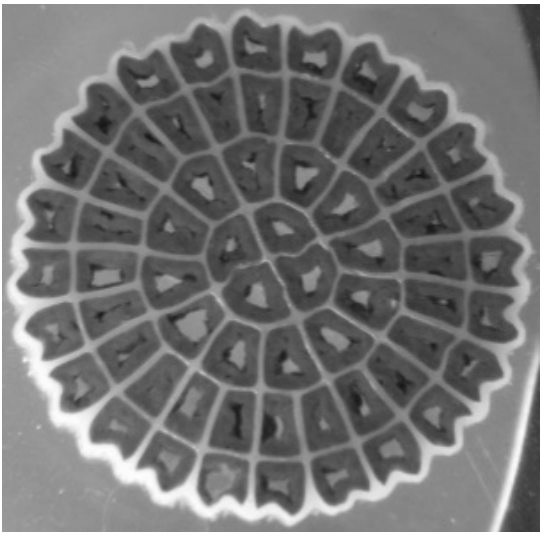


Figure 1.25. *Biofilm on AnoxKaldnes™ substrate*

The IFAS process comprises two stages during which bacteria develop in aerobic and anaerobic zones. AOB bacteria are present in free cultures (activated sludge), while anammox bacteria are present on the biofilm.

The operating parameter of the Anita™ Mox process is the O_2 concentration, which influences the activity of AOB and anammox bacteria, and therefore the efficiency of deammonification. The removal rates of NH_4^+ with MBBR amount to 1.2 kg $N.m^{-3}$, whereas in IFAS mode, they reach 3 kg $N.m^{-3}$.

The process has opened up attractive new solutions for nitrogen removal applications due to the ease of modernizing the IFAS process in activated sludge systems with existing clarifiers.

In 2010, after two years of pilot tests in France, Denmark and Sweden, the Anita™ Mox process was implemented on an industrial scale at the Sjölunda wastewater treatment plant near Malmö in Sweden (550,000 EH). Today, it processes 200 kg of

nitrogen per day from sludge digestion. This unit also serves as a “biofarm” to produce substrates with a mature and efficient biofilm.

This strategy was implemented to accelerate the start-up of other Anita™ Mox industrial units at the wastewater treatment plants of Sundets in Växjö, Sweden; Holbaek and Grindsted in Denmark; and even recently other facilities in the United States.

In France, the recent development of anaerobic sludge digestion to produce biogas and move towards energy self-sufficiency makes the treatment of nitrogenous wastewater through these new biological processes increasingly acceptable and feasible.

Anita™ Mox can also be applied to other effluents high in NH_4 and with little BOD, such as landfill leachate, industrial effluents or leachate from UASB-type anaerobic processes.

Essentially, Anita™ Mox is a process dedicated to the treatment of NH_4 in return supply lines, simultaneously implementing:

– the aerobic nitritation step ($NH_4 \rightarrow NO_2$; nitrifying bacteria);

– the anoxic deammonification stage without a carbon source ($NH_4 + NO_2 \rightarrow N_2$; anammox bacteria).

Compared to conventional nitrification/denitrification, the process allows:

– a 60% reduction in O_2 requirements;

– the complete removal of the need for carbon sources;

– an 80% reduction in sludge production.

The Anita™ Mox process was adapted into the synergistic interaction of this type of biofilm, composed primarily of anammox bacteria, with free biomass (conventional activated sludge) within the MBBR. This configuration requires the implementation of a clarifier downstream of the MBBR to recirculate the activated sludge back into the reactor. This is called the Integrated Fixed-Film Activated Sludge (IFAS) configuration. This difference in the spatial organization of bacteria allows the Anita™ Mox process in the IFAS configuration to be two to three times more compact than the MBBR configuration despite the addition of equipment such as the clarifier.

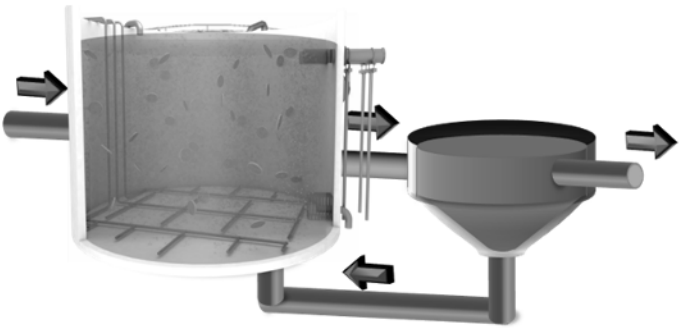


Figure 1.26. *Diagram of the Anita™ Mox process in IFAS configuration with its clarifier*

Site	Country	Capacity	Type of effluent	Date
Sjölunda (Malmö)	Sweden	200 kg N.d^{-1}	Digestion	2010
Sundets (Växjö)	Sweden	430 kg N.d^{-1}	Digestion	2012
Holbaek	Denmark	120 kg N.d^{-1}	Digestion	2012
Grindsted	Denmark	110 kg N.d^{-1}	Digestion	2013
James River (Newport)	United States	250 kg N.d^{-1}	Digestion	2013
South Durham	United States	330 kg N.d^{-1}	Digestion	2014
Egan (Chicago)	United States	940 kg N.d^{-1}	Digestion	2016
Mars Candy	Poland	340 kg N.d^{-1}	F&B return after AnMBR	2015
Starch	Germany	300 kg N.d^{-1}	F&B return after UASB	2016
Viikinmäki (Helsinki)	Finland	320 kg N.d^{-1}	Municipal sidestream	2016
Sobacken (Boraas)	Sweden	800 kg N.d^{-1}	Municipal sidestream	2017
Denver Metro	United States	3,000 kg N.d^{-1}	Municipal sidestream	2018
Tomahawk (Johnson)	United States	–	Municipal sidestream	2018

Table 1.15. *Anita™ Mox (Veolia) references*

1.8.15.3. *Paques process*

Since Paques launched the first biological nitrogen filtration system in 2002, ANAMMOX® has become the most widely used nitrogen removal system in the world; it offers a treatment capacity of over 160,000 kg of nitrogen per day. The ANAMMOX® conversion is an elegant shortcut in the natural nitrogen cycle.

Anammox bacteria convert ammonium (NH_4^+) and nitrite (NO_2^-) into nitrogen (N_2) gas, which is environmentally neutral. Paques's patented ANAMMOX® technology combines the anammox process with partial nitritation (conversion of ammonium to nitrite). The ANAMMOX® process works with naturally cultivated concentrated granular sludge, creating optimal conditions for microbial synergy.

1.8.15.4. *Sharon® process*

Initially developed by a group of researchers at Delft University in the Netherlands, the Sharon® process is a two-step process. Its acronym is derived from the overall name: Single-reactor System for High Ammonia Removal Over Nitrite. The Sharon® process is a biological system for treating effluents with high ammoniacal nitrogen content. It is based on the selection of a specific microorganism culture, with conditions defined by various process parameters such as sludge age, pH and temperature, in order to implement so-called partial nitrification or a nitrate shunt. The aim is to treat ammonia nitrogen with aerobic nitritation ($N\text{-}NH_4 \rightarrow NO_2^-$) followed by anaerobic denitrification ($NO_2^- \rightarrow N_2$), the classic nitrate stage ($NO_2^- \rightarrow NO_3^-$). The treatment system includes an anoxic tank for denitrification (non-aerated but continuously agitated) and an aerated and agitated tank for nitritation located outside the former. The stored effluent (at 30–40°C) temporarily held in the buffer tank is cooled in a heat exchanger and then fed into the anoxic reactor. The sludge–effluent mixture then passes through the aerated reactor via a wall pump and returns to the anoxic tank by overflow. The hydraulic retention time of the system is identical to the sludge age, as the sludge neither settles nor recirculates. For process optimization, the tank pH can be regulated by adding sodium hydroxide. Phosphorus rebalancing is achieved by adding phosphoric acid, and organic carbon is supplied in the form of methanol for denitrification. According to the manufacturer, the Sharon® process and its nitrate shunt should help reduce energy costs since the O_2 consumption is 25% lower during nitrification and 40% for carbon during denitrification. It should reduce biological sludge production by 25% by decreasing the number of steps in the chemical reactions. Finally, the denitrification rate is also 1.5 to 2 times higher with partial nitrification compared to conventional nitrification.

Over the past decade, an increasing number of researchers have begun to focus on the traditional anammox process. However, the traditional anammox process faces more practical challenges.

Firstly, there is little focus on NH_4^+ in municipal wastewater; it is generally 20 to 60 mg-N L^{-1} (Gilbert et al. 2014). The lack of substrate inhibits the activity of ammonia-oxidizing bacteria and anammox bacteria, and the lower concentration of free ammonia and free nitrous acid makes it more difficult to inhibit the activity of NOB.

Secondly, the temperature of municipal wastewater is low in winter (generally < 15°C), which considerably inhibits the activity of anammox bacteria (Gilbert et al. 2014). Furthermore, the GDP growth rate is faster than that of AOB at low temperatures, leading to substrate competition between anammox and NOB.

Thirdly, the carbon-to-nitrogen (C/N) ratio in the main influent is higher. A high C/N ratio promotes the proliferation of heterotrophic bacteria, intensifying competition between heterotrophic and anammox bacteria for the NO_2^-, reducing the overall nitrogen removal efficiency.

Most importantly, the slow growth rate of anammox bacteria and the interference of complex microbial communities have become two bottlenecks with the primary anammox process. Indeed, the growth rate of Anammox bacteria is slow. It is only 0.002 to 0.007 h^{-1}, while the maximum growth rates representative of AOB, NOB and denitrifiers are respectively 0.09 h^{-1}, 0.06 h^{-1} and 0.26 h^{-1}. Consequently, this slow growth rate results in a long start-up time and a low nitrogen removal rate. The anammox process requires sufficient nitrite acting as an electron acceptor. However, nitrogen in municipal wastewater exists primarily in the form of NH_4^+; it is generally necessary to combine partial nitrification with anammox. First, ammonia is oxidized to nitrite by AOB bacteria. Then, the remaining ammonia is oxidized to nitrogen gas by the accumulated nitrite through the action of anammox bacteria.

1.8.16. *Applications*

A large number of configurations incorporating anammox bacteria have been proposed in treatment processes. Figures 1.27 to 1.29 present three of them.

1.8.16.1. *Mainstream configuration*

The anammox system is installed in a continuous configuration, on the same line as the main treatment facilities. Conventional treatment removes organic carbon. Nitritation is then carried out in conjunction with the anammox system.

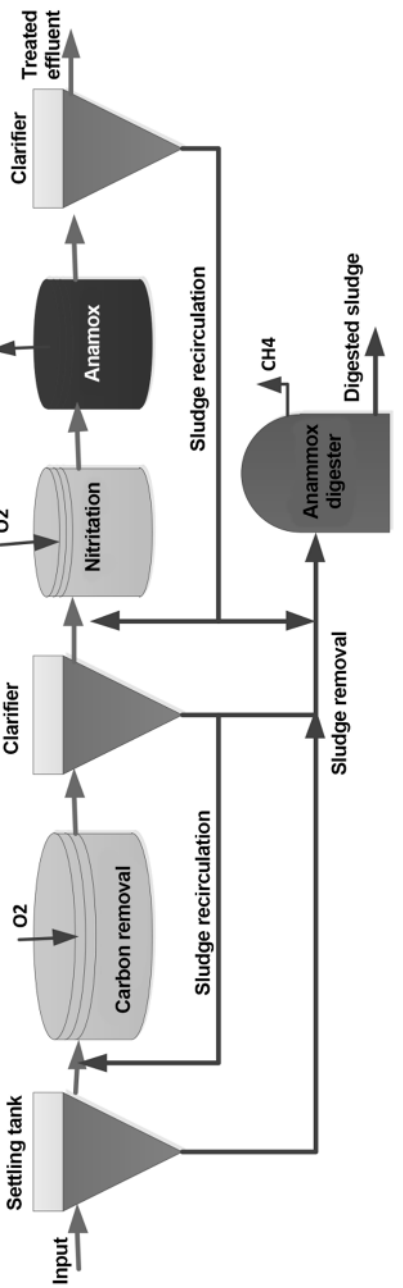


Figure 1.27. *Anammox installed in the main process*

1.8.16.2. *Sidestream configuration*

Anammox treats high NH_4 concentrations present in the digester supernatant rich in inlet effluent or cells from the DN.

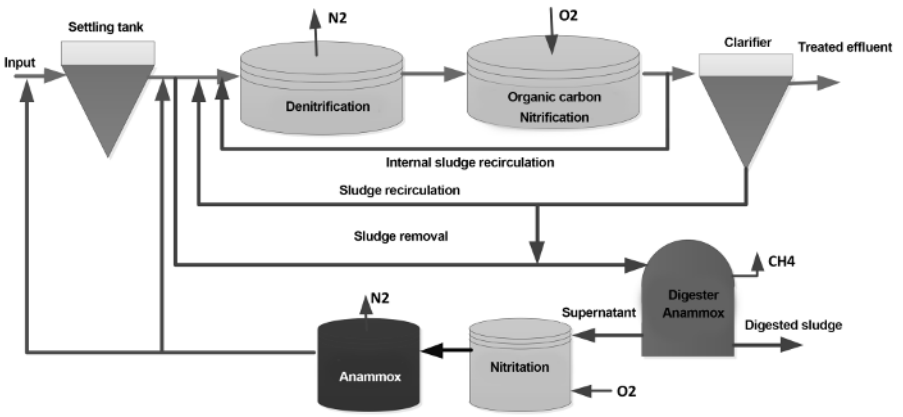


Figure 1.28. *Anammox installed on the secondary line*

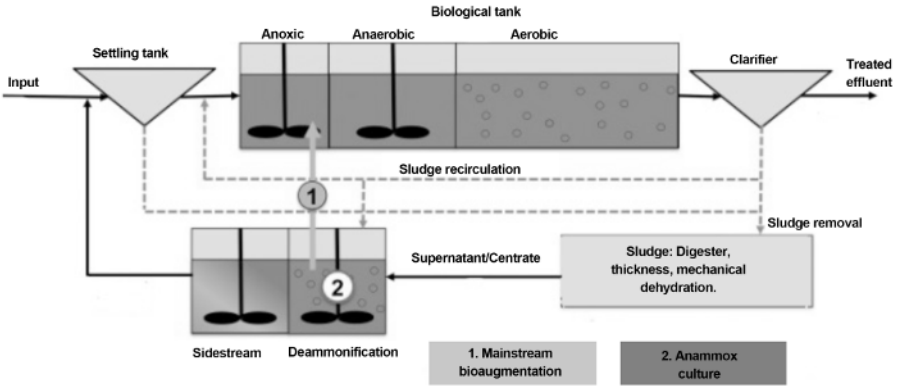


Figure 1.29. *Mainstream and sidestream configurations for part of returning sludge*

Figure 1.29 shows the solution that prevents potential failure of the anammox reactor (second stage). Lateral deammonification can be used as a solution for nitrogen treatment, as well as for cultivating anammox for bio-augmentation of the biological tank. A rapid increase in the inlet nitrogen load (i.e. due to a failure of the

sidestream deammonification process) can lead to a decrease in the quality of the main reactor effluent, particularly at cold temperatures. The additional AOB mass increases the nitrogen removal capacity of the main reactor and protects against effluent quality degradation during a period of increased load or a sudden loss of biomass.

1.9. MABR: a new opportunity

Like activated sludge, wastewater treatment in a membrane aerated biofilm reactor (MABR) uses microorganisms to nitrify and denitrify wastewater, but the MABR uses a spirally wound, self-aspirating membrane with a large surface area, which passively supplies oxygen instead of manually pumping air into the water. This configuration offers several advantages. Aeration is carried out at a pressure close to atmospheric pressure, virtually eliminating the costly air compression that accounts for 40–60% of energy consumption in existing processes. A natural, resistant layer of microorganisms forms on the oxygen-rich surface of the membrane, where bacteria can use oxygen most efficiently. In contrast, with conventional bubble aeration, most air bubbles rise to the surface before microorganisms can use them (Ni et al. 2016).

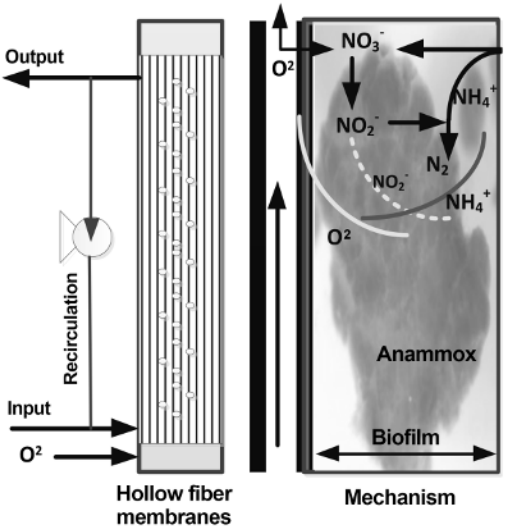


Figure 1.30. *Basic MABR operating principle diagram*

Conditions in the rest of the MABR tank remain anoxic, so anaerobic denitrifying bacteria can do their work in the same reservoir as aerobic bacteria. This

simultaneous nitrification–denitrification (SND) process allows for a smaller footprint compared to traditional processes that require multiple chambers.

1.10. Conclusion

Several low-carbon nitrogen removal processes have been developed, including nitritation–denitrification, anammox-regulated processes, bioelectrochemical processes, biofilm-on-membrane association with O_2 diffusion and autotrophic denitrification.

Identifying which biological processes are best for nitrogen removal in municipal wastewater treatment is important for future development, as there are various emerging processes that can have a significant impact on resource use and climate change. In theory, processes using anammox surpass all others in terms of net energy production while significantly reducing sludge treatment requirements. However, the efficiency and reliability of anammox treatment have not yet been demonstrated, even though the Veolia MBBR and IFAS installations perform very well. Certainly, the difficulties encountered include the slow growth of organisms, the effects of temperature and the challenges of balancing oxygen supply with the significant diurnal variations in nitrogen that occur in the main flow. Insufficient oxygen leads to low ammonia removal efficiency, while excessive oxygen results in excessive nitrate production. However, given the significant potential environmental benefits of this novel combination of processes, research aimed at accelerating large-scale development appears to be an urgent need.

Bioelectrochemical processes are limited by the low wastewater conductivities, and low applied loads restrict their large-scale application. Autotrophic denitrification induced by sulfur and its compounds is only viable for moderate removed loads (1 kg-N.m^{-3} d^{-1}). However, it generates harmful chemicals that require downstream disposal to avoid negative environmental impacts. Overall, nitrogen removal processes using anammox offer the best alternatives to nitrification/denitrification in terms of COD demand, simplicity and process performance.

There are many aerobic and anaerobic biological processes and different treatment schemes for transforming organic matter and/or removing nitrogen from domestic wastewater. Significant reductions in oxygen requirements and no need for organic matter for nitrogen reduction are often cited as advantages of using the newer anammox organism approach for nitrogen removal rather than the traditional nitrification/denitrification method, the most common one used today. However, treatment schemes differ, and for some, the suggested benefits may not be acceptable. When nitrification/denitrification is used, an anoxic tank is commonly used at the beginning of the process, and the nitrates formed by the nitrification stage are then

recycled to the anoxic tank, which benefits from the presence of organic matter in untreated wastewater.

This significantly reduces oxygen requirements and the need to add organic matter (methanol) when denitrification is carried out at the end of the process.

So, when are such statements correct and when are they not? What factors in the wastewater composition, regulatory requirements and treatment scheme alter the most appropriate treatment process? To help make such judgments in different circumstances, the stoichiometry of the various biological processes involved and the different treatment approaches used must be determined and compared.

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