sciendo

Mathematical Modelling in Civil Engineering
Vol. 16-No. 3: 55-65-2021
Doi: 10.2478/mcee-2021-0015

KINETICS AND CHEMISTRY OF NITRIFICATION PROCESS – A REVIEW

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Abstract: Nitrogen is an important nutrient for the survival of all living organisms, as its chemical binding properties allow to create structures from which proteins are built. In nature, nitrogen is found in many forms and its transformation from one form to another is described by the nitrogen cycle. Ammonium is a form of nitrogen that has no direct effects on human health, but its presence in water supply systems may determine bacterial growth in the distribution networks and ultimately can generate degradation of the water quality. Its concentrations in wastewater and water intended for human consumption can be reduced by the biological process of nitrification. The purpose of this paper is to review the equations and the kinetics of the biological nitrification process.

Keywords: biological process, growth rate, stoichiometric equations, kinetics

1. Introduction

Nitrogen is a critical nutrient for the survival of all living organisms, as its chemical binding properties allow it to create structures such as DNA, RNA and amino acids, structures from which proteins are built [1] [2].

Nitrogen was officially discovered in 1772 by Scottish researcher Daniel Rutherford. At the same time, nitrogen or “burnt air” (the name at the time) was studied by Carl Wilhelm Scheele, Henry Cavendish and Joseph Priestley, but the official merit for the discovery of nitrogen was earned by Daniel Rutherford, who was the first to publish the results [3].

The name "Nitrogen" was invented in 1790 by the French chemist Jean Antoine Claude Chaptal. He originally called it "Nitrogen", a reference to nitrate (potassium nitrate), which was known to contain nitrogen [3].

In nature, nitrogen is found in many forms, and its transformation from one form to another is described by the nitrogen cycle [4].

The nitrogen circuit is the biogeochemical cycle by which nitrogen is transformed into multiple chemical forms as it circulates through atmospheric, terrestrial and marine ecosystems. Nitrogen conversion can be accomplished through both biological and physical processes.

Nitrogen enters the living world through bacteria and other single-celled prokaryotes, which transform atmospheric nitrogen N_2 into biologically usable forms. Some species of nitrogen-fixing bacteria live freely in soil or water, while others are beneficial symbionts that live inside plants [5].

Thus, nitrogen undergoes multiple transformations in the ecosystem, changing from one form to another, as organisms use it for growth and, in some cases, for energy generation. The major transformations of nitrogen are: fixation, nitrification, ammonification, assimilation and denitrification [1]. The following figure shows a schematic of the nitrogen cycle in nature.

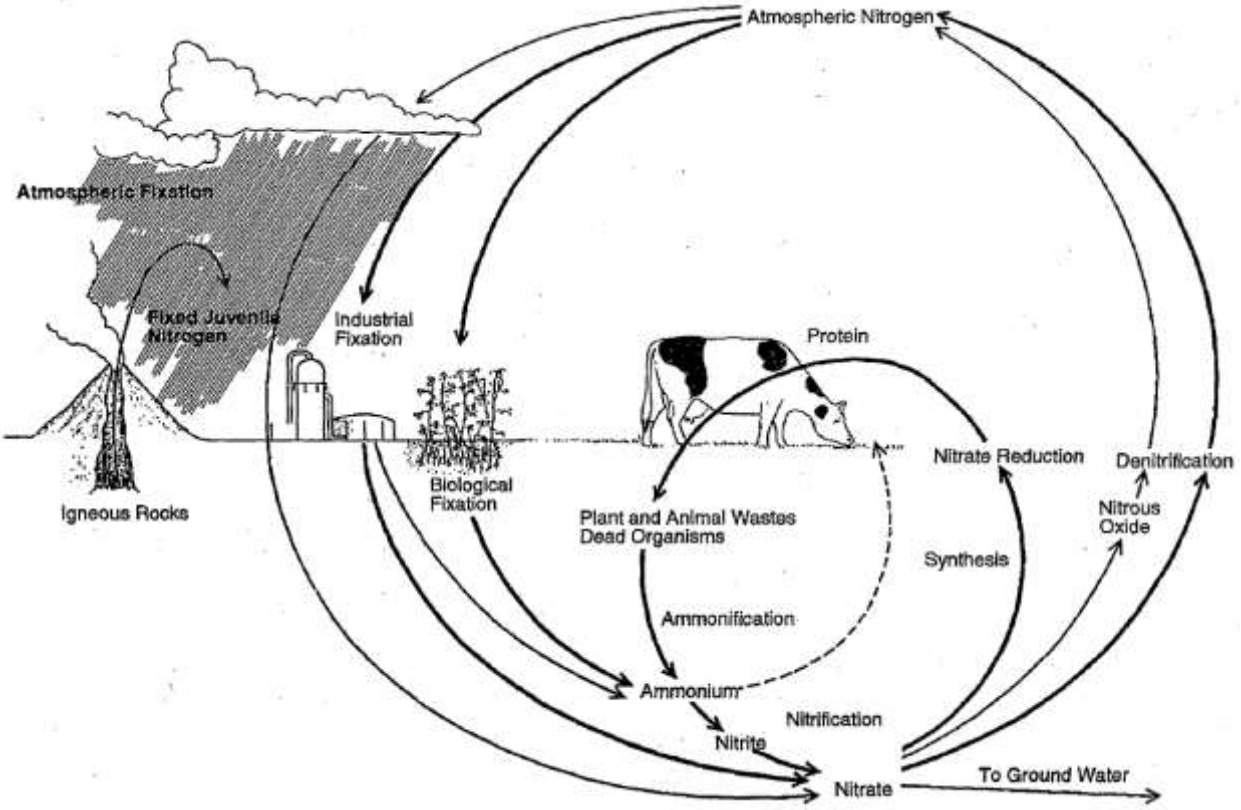

Fig. 1 - The nitrogen cycle in nature [4].

Ammonium and nitrates are the primary sources of nitrogen for plants, but ammonium is the first element absorbed when plants are nitrogen deficient [6].

Ammonium has no direct effects on human health, but its presence in water supply systems may generate development of bacteria in the distribution networks and ultimately degradation of water quality [7].

Current technologies for reducing ammonium in water are represented by physical, chemical and biological processes. But the processes that can be used successfully and with low energy costs for both water treatment and wastewater treatment are represented by biological processes of nitrification and denitrification.

All biological growth processes require nitrogen in a form or other for the synthesis of cellular proteins and nucleic acids. Microorganisms can use a range of nitrogen compounds for these syntheses under a variety of conditions, which means in some cases the oxidation state of nitrogen is changed while in others it is not. There are others transformations of nitrogen in which the nitrogen containing compound is used as a source of oxygen or is itself oxidized to provide energy for synthetic reactions [8].

Nitrification is an important microbiological reaction of nitrogen [9]. This biological process is used to reduce the concentrations of ammonium in water to nitrates.

2. Fundamentals of the biological nitrification process

Nitrification is the biological process of oxidizing ammonium to nitrate with an intermediate form of nitrite. Nitrification is usually performed by the bacterial species Nitrosomonas and Nitrobacter. Both species of bacteria are classified as autotrophic organisms [10] [11] [12].

These bacteria differ from heterotrophic bacteria because they use energy for growth, from the oxidation of inorganic nitrogen compounds in water and not from the oxidation of organic matter, as in the case of heterotrophic bacteria. Another feature is the use of carbon dioxide in cell

synthesis, resulting in no need to add organic carbon to water [13] [4]. However, there is evidence that Nitrobacter can also use organic carbon as an energy source [12].

The two species of bacteria are distinguished from each other by the ability to oxidize only certain nitrogen compounds. Nitrosomonas is limited to the oxidation of ammonium to nitrite and Nitrobacter is limited to the oxidation of nitrite to nitrate [10] [11] [12].

2.1. Biochemical metabolism

At the biochemical level, the process involves more than a continuous oxidation of ammonium to nitrate, because many intermediate reactions and enzymes are involved, but the most important is to know the response of bacteria to environmental conditions, in order to achieve systems in which they can perform effectively the metabolic activity [13] [12].

Since complete nitrification is a sequential reaction, treatment systems must be designed to provide an environment suitable for the growth of both nitrifying bacteria (Nitrosomonas and Nitrobacter) [4] [12].

2.2. Stoichiometric equations

The stoichiometric equations of nitrification for Nitrosomonas and Nitrobacter are [4] [13] [12] [14]:

$$NH_4^+ + 1.5O_2 -> 2H^+ + H_2O + NO_2^- \quad (1)$$

$$NO_2^- + 0.5O_2 -> NO_3^- \quad (2)$$

The equation of ammonium oxidation to nitrate made by Nitrosomonas and Nitrobacter is [9] [4] [12] [13]:

$$NH_4^+ + 2O_2 -> NO_3^- + 2H^+ + H_2O \quad (3)$$

Compared to heterotrophic bacteria, the growth of nitrifying bacteria is very slow, and the yield of cells per unit of oxidized energy is low [12].

The oxidation reaction of ammonium to nitrate provide the energy needed for microorganisms (bacterial) growth. These assume that the empirical formulation of bacterial cells is $C_5H_7NO_2$. Assuming that the empirical formulation of biomass is $C_5H_7NO_2$, the stoichiometric equations for Nitrosomonas and Nitrobacter will have the following forms [4] [12] [13]:

$$13NH_4^+ + 15CO_2 -> 10NO_2^- + 3C_5H_7NO_2 + 23H^+ + 4H_2O \quad (4)$$

$$10NO_2^- + 5CO_2 + NH_4^+ + H_2O -> 10NO_3^- + C_5H_7NO_2 + H^+ \quad (5)$$

Bacterial cells grow by combining energy-producing reactions with those involving cell synthesis. Thus, cell synthesis can be described by combining the equations for energy efficiency and cell synthesis. The efficiency of bacteria in converting released energy into biomass dictates how these equations are combined. Efficiency can be measured in terms of observed yield, expressed as cell mass produced/mass of substrate used. This yield coefficient is normally specified as the mass of VSS (volatile suspended solids) produced/mass of ammonium or nitrite oxidized [4].

The yield values calculated from the theoretical energy release ratios are 0.29 g VSS/g NH_4^+-N and 0.084 g VSS/g NO_2^--N [15].

The yield values observed in the experiments are lower. Yield values vary from 0.06 to 0.13 for Nitrosomonas, but at 25°C values of 0.04-0.1 have been obtained. Yield value for Nitrobacter is lower, 0.02 but higher values between 0.04-0.07, have been obtained for growth in continuous culture [8].

Observed yields may be lower than theoretical yields because a fraction of the free energy released by oxidation is diverted to microbial maintenance functions and will vary with changing environmental conditions and with changes in the microbial cells rate growth, in part accounting

for the range of observed yield values. The total yield of nitrifiers, when considering nitrification as a single-step process from ammonium to nitrate is 0.06-0.20 g VSS/g NH_4^+-N oxidized [4].

If yields of 0.08 g VSS/g NH_4^+-N and 0.05 g VSS/g NO_2^--N, respectively, are to be considered, then the synthesis equations for Nitrosomonas and Nitrobacter are the following [4]:

$$1.0NH_4^+ + 1.44O_2 + 0.0496CO_2 -> 0.01C_5H_7O_2N + 0.99NO_2^- + 0.97H_2O + 1.99H^+ \quad (6)$$

$$1.0NO_2^- + 0.00619NH_4^+ + 0.031CO_2 + 0.0124H_2O + 0.5O_2 -> 0.00619C_5H_7O_2N + 1.0NO_3^- + 0.00619H^+ \quad (7)$$

Combining the two equations, the equation that describes the completed nitrification will be obtained [4]:

$$1.0NH_4^+ + 1.89O_2 + 0.0805CO_2 -> 0.0161C_5H_7O_2N + 0.952H_2O + 0.984NO_3^- + 1.98H^+ \quad (8)$$

Stoichiometric coefficients indicate that in order to oxidize ammonium, the nitrification process needs oxygen, produces a small amount of biomass and substantially reduces alkalinity by producing hydrogen ions [4].

In current practice, the accepted values for oxygen, alkalinity and yield coefficient, used in the dimensioning of systems based on nitrification, are:

- Oxygen – 4.6 g of oxygen needed/g NH_4^+-N;
- Alkalinity – 7.1g alkalinity (as $CaCO_3$) destroyed/ g NH_4^+-N;
- Yield coefficient – 0.1 g VSS produced as nitrifiers)/g NH_4^+-N.

3. Nitrification kinetics and the factors that influence nitrification

The kinetics of the nitrification process can be considered as the study of the factors that influence and explain the rates of nitrification reactions. Ammonium removal in the nitrification process takes place by microbial synthesis or growth and oxidation [4].

In cases where ammonium removal is required, the nitrification kinetics will govern the design of the aerobic area of the biological reactor, because nitrification bacteria have slower growth rates than heterotrophic bacteria, requiring a longer solids retention time [14].

The following subchapters will present the kinetic equations that describe the growth rate of nitrifying bacteria during ammonium oxidation, equations that take into account the impact of factors such as dissolved oxygen, pH, temperature and other inhibitory factors [4] [13].

3.1. Biomass growth and ammonia utilization

There is still insufficient knowledge about the mechanisms of biomass growth to propose an equation that accurately characterizes biomass growth, but researchers have observed the effects of various factors on growth and then tried to adapt the empirical equations to their observations [16].

The equation that best describes the biomass growth is the equation proposed by Monod [9] [4] [12] [13] [16].

$$\mu = \mu_{max} \frac{S}{K_S + S} \quad (9)$$

where,

μ – specific growth rate/rate of microorganisms [day^{-1}];

μ_{max} – maximum specific growth rate/rate of microorganisms [day^{-1}];

K_S –half-saturation or half-speed coefficient (equivalent to the concentration of the substrate which limits growth to half of the maximum specific growth rate) [mg/l];

S – growth-limiting substrate concentration [mg/l].

Although the Monod equation is the most widely accepted description of microbial growth kinetics, it has some theoretical deficiencies, and these are particularly evident when the term is used to describe processes that may involve several substrate limiting conditions (e.g., microbial growth limited by ammonium or oxygen in transient conditions relative to equilibrium) and associated groups of multiple organisms [4].

3.1.1. The relationship between the growth rate and the oxidation rate

The rate of ammonium oxidation is controlled by the Nitrosomonas growth and is related to this increase by the Nitrosomonas yield coefficient, according to the following equation:

$$q_N = \frac{\mu_N}{Y_N} = q_{max\,N}\frac{N}{K_N+N} \quad (10)$$

where,

q_N – ammonium oxidation rate [g NH_4^+-N oxidized/g VSS/day];

$q_{max\,N}$ – maximum ammonium oxidation rate [g NH_4^+-N oxidized/g VSS/day];

μ_N – specific growth rate of Nitrosomonas [day^{-1}];

Y_N – organism yield coefficient [g Nitrosomonas grown (VSS)/g NH_4^+-N oxidized];

N – NH_4^+-N concentration [mg/l];

K_N – half-saturation coefficient for Nitrosomonas [mg/l NH_4^+-N].

3.1.2. The relationship between the growth rate and the retention time of solids

Since the microorganisms' growth rate is low, it takes a sufficient amount of solids retention, usually called the sludge age. This retention time is especially important to maintain a population of ammonium nitrifying bacteria. The retention time (θ_c) is defined according to the following equation [4]:

$$\theta_c = \frac{\text{total microbial mass in the treatment system}}{\text{total quantity of biological mass wasted daily}}\ [\text{day}] \quad (11)$$

At steady state, the solids leaving the system will be equal to the solids produced, that means the growth rate and solids retention time of the microorganisms in the system are defined according to the following equation [4]:

$$\frac{1}{\theta_c} = \mu_N - b_N = \mu'_N \quad (12)$$

where,

μ'_N – net specific growth rate of nitrifiers [day^{-1}];

b_N – endogenous degradation coefficient for nitrifiers [day^{-1}].

Usually the endogenous degradation coefficient for nitrifiers is negligible and is considered equal to 0, resulting in the previous equation will have the following form:

$$\frac{1}{\theta_c} = \mu_N \quad (13)$$

3.2. Kinetic rate constants for temperature and nitrogen concentration

There are studies that have shown that the nitrification process is strongly dependent on temperature. According to existing studies, both the microorganisms' growth rate and the half-saturation coefficient are affected by temperature and can be described according to the following equations [12] [13].

$$K_N = 10^{0.051T-1.158} \text{ [mg/l N]} \quad (14)$$

$$\mu_{\max N} = 0.47e^{0.098(T-15)} \text{ [day}^{-1}\text{]} \quad (15)$$

where,

T – temperature [^oC]

$\mu_{max\ N}$ – maximum growth rate of Nitrosomonas [day^{-1}]

According to data from the literature, accurate quantification of the effect of temperature is difficult, but according to existing studies, it can be said that an increase in temperature by about 10 ^oC, leads to doubling the speed of microorganisms' growth. The maximum specific growth rate of Nitrosomonas increases by about 9.5 percent for each 1 °C increase in temperature. Given that the temperature in an aerobic system can vary from 8 °C to 30 °C over the course of a year, tests were performed at different temperatures to determine the impact of temperature on the nitrification process and according to the results obtained, as the temperature decreases, the efficiency of nitrification at any sludge age, decreases, except for the very high values of the sludge age. In order to maintain the same level of nitrification during winter temperatures, the sludge age may need to increase substantially. If a treatment plant does not have the capacity to increase the sludge age sufficiently during the winter, nitrification may fail partially or completely. The minimum expected temperature and the minimum value for the maximum growth rate must be taken into account in the design if nitrification is desired throughout the year [17].

A study showed that although both nitrite and nitrate formation are strongly inhibited at temperatures of 10 °C or less, the inhibitory effect of low temperature was higher for Nitrobacter than for Nitrosomonas; this was evident from the high nitrites' accumulation at low temperatures. The thermal death point of a pure Nitrosomonas culture was between 54 and 58 ^oC, and at below 4 ^oC almost no nitrifying bacteria growth was found [12].

Suspended growth cultures are more sensitive to temperature changes than bacteria attached to a support medium [12].

The nitrification process takes place at temperatures between 4 and 45 ^oC, with an optimal temperature of 35 ^oC for Nitrosomonas and an optimal temperature of 35-42 ^oC for Nitrobacter [10] [4].

3.3. Effects of pH and alkalinity on kinetics

When the equation describing the complete nitrification' process is written in the context of the carbonic acid system, there is a substantial reduction in alkalinity [10] [13].

According to the literature, in a biological system and with a pH between 5 and 8, the equilibrium pH of the biological treatment system is dictated by the concentration of alkalinity and by the carbon dioxide present in the system [4].

As the nitrification process reduces the HCO_3^- level and increases the H_2CO_3 level, the pH tending to decrease. This effect is mediated by stripping of carbon dioxide in water by aeration and therefore the pH is often increased. If carbon dioxide is not removed from the liquid, the pH may be lowered to 6 [12].

The following equation shows the effect of pH on biomass growth rate [12] [13]:

$$\mu_N = \mu_{\max N}\left(1 - 0.833(7.2 - pH)\right) \text{ [day}^{-1}\text{]} \quad (16)$$

For pH levels between 7.2 and 8.0 the growth rate is assumed to be constant. This expression was developed for combined carbon oxidation-nitrification systems, but its application to separate stage of nitrification systems is probably conservative [13].

The nitrification rate will slow down significantly below a pH of about 6.8. According to the literature, nitrification rates at a pH of 6.0 can be only about 20% of those with a pH of 7.0. Thus,

treatment plants where raw water enters with low alkalinity may experience periods of low nitrification and high levels of ammonium in the treated water [14].

Due to the effect of pH on the nitrification rate, it is particularly important that the water be sufficiently alkaline to balance the acid produced by nitrification, and if there is not enough alkalinity in the water, a pH correction must be made [12] [13].

The theoretical ratio between alkalinity and oxidized ammonium is 7.1 mg $CaCO_3$/mg NH_4^+-N oxidized [4] [12].

For the proper functioning of the nitrification process, it is recommended that the pH be maintained in the range of 6.5-8, with an optimal pH of 7.2 [10] [4].

3.4. Effects of dissolved oxygen on kinetics

In drinking or waste water treatment systems, the concentration of dissolved oxygen has a significant effect on the nitrifying bacteria growth rates and on the nitrification process.

The Monod relation can be used to model the effect of dissolved oxygen on the bacteria growth by considering oxygen as a growth-limiting substrate, as follows [12] [13]:

$$\mu_N = \mu_{max\,N} \frac{DO}{K_{o2}+DO} \tag{17}$$

where,

DO – dissolved oxygen [mg/l];

K_{O2} – half-saturation constant for oxygen [mg O_2/l].

Over time, numerous experimental tests have been performed to determine the oxygen saturation half-constant and values between 0.15 and 2.0 mg/l have been obtained. However, according to literature, the half-saturation constant of oxygen is considered 1.3 mg/l [13].

In engineering calculations, it is considered that a dose of 4.6 mg/l dissolved oxygen per mg/l of oxidized ammonium is required, but at the same time the need for dissolved oxygen must be taken into account for the oxidation of other compounds present in water [12].

However, for optimal operation of the nitrification process it is recommended that the minimum level of dissolved oxygen concentration be 2 mg/l. The presence of oxygen is a mandatory requirement for all species of nitrifying bacteria, and in the absence of oxygen, ammonium oxidation reactions cease or proceed at a slow pace [10].

3.5. Combined kinetic expressions

In the previous subchapters, the effects of ammonium and dissolved oxygen concentrations, pH and alkalinity on nitrification processes were presented separately, but in real life these parameters simultaneously affect the nitrification process.

The combined effect of several limiting factors on biological growth can be introduced as a product of Monod type factors, according to the following equation [13]:

$$\mu_N = \mu_{max\,N}\left(\frac{L}{K_L+L}\right)\left(\frac{N}{K_N+N}\right)\left(\frac{P}{K_P+P}\right) \tag{18}$$

where,

L - the concentration of substance L that limits growth [mg/l];

N - the concentration of substance N that limits growth [mg/l];

P - the concentration of substance P that limits growth [mg/l];

K_L, K_N and K_P, – half-saturation constant for substances L, N and P [mg/l].

Starting from this approach, the equation that would describe the combined kinetic expression for the nitrifying bacteria growth would take the following form [12] [13]:

$$\mu_N = \mu_{max\ N}\left(\frac{N}{K_N+N}\right)\left(\frac{DO}{K_{o2}+DO}\right)(1-0.833(7.2-pH)) \quad (19)$$

where,

$\mu_{max\ N}$ – maximum growth rate of nitrifiers for a known temperature and pH [day^{-1}].

Integrating in equation 19, equations 14 and 15, the following form is obtained [12] [13]:

$$\mu_N = [0.47e^{0.098(T-15)}]\left[\frac{N}{10^{0.051T-1.158}+N}\right]\left[\frac{DO}{1.3+DO}\right][1-0.833(7.2-pH)] \quad (20)$$

Taking into account the previous relations, then the ammonium elimination rate can be defined as [13]:

$$q_N = \frac{\mu}{Y_N} = \frac{\mu_{max\ N}}{Y_N}\left(\frac{N}{K_N+N}\right)\left(\frac{DO}{K_{o2}+DO}\right)(1-0.833(7.2-pH)) \quad (21)$$

Knowing the temperature, pH and concentrations of nitrogen (from ammonium) and dissolved oxygen, with the previous two equations, can determine the rate of biological growth and ammonium reduction rate [13].

The biggest problem in the analysis of nitrifying bacteria growth rate, is the estimation of nitrifier concentration for determination of the specific growth rate, of the yield coefficient and of the constant saturation [12].

3.6. Effects of inhibitors

Nitrifying bacteria are susceptible to a wide range of organic and inorganic inhibitors. However, nitrifying bacteria can adapt to many inhibitory compounds when the inhibitors are constantly present in the raw water, compared to the occurrence of high concentrations due to accidental discharges into the water source, untreated or insufficiently treated industrial wastewater. Inhibition may occur by interfering with the general metabolism of the cell or with primary oxidative reactions. However, more important than the differentiation of the inhibition mechanism is the need to establish a methodology for assessing the potential or occurrence of nitrification inhibition [4].

Over time, various methodologies have been proposed to assess the potential for inhibition of nitrification by organic inhibitors, and these methodologies have led to the identification of a list of industrial organic pollutants and their concentrations at which nitrification is inhibited by at least 50%. These pollutants are presented in the following table [4].

Table 1

Industrial organic compounds that inhibit the nitrification process [4]

Item	Polluant	The concentration of the pollutant at which it generates the inhibition of at least 50% of the nitrification [mg/l]
1	Acetone	2,000
2	Carbon disulfide	38
3	Chloroform	18
4	Ethanol	2,400
5	Phenol	5.6
6	Ethylenedlamine	17
7	Hexamethylene	85
8	Aniline	<1
9	Monoethanolamine	<200

Certain inorganic compounds, including specific metals, are inhibitory to nitrifying bacteria. It is considered that 10-20 mg/l of heavy metal can be tolerated due to low ionic concentrations at pH values of 7.5-8.0. Inorganic compounds identified as potential inhibitors are: zinc, free cyanide, perchlorate, copper, mercury, chromium, nickel, silver, cobalt, thiocyanate, sodium cyanide, sodium azide, hydrazine, sodium cyanate, potassium chromate, cadmium, arsenic (trivalent), fluorine and lead [4].

Like other microorganisms, nitrifying bacteria can grow at their maximum growth rate when optimum environmental factors can be obtained in an environment without any toxic substances [12].

3.7. Bacteria adaptation to environmental conditions

The growth kinetics of nitrifying bacteria, presented in the previous subchapters, are based on pure cultures of nitrifying bacteria, but such systems with pure cultures do not exist in reality. This has effects on the sizing of nitrification systems [13].

The ratio of the feed biodegradable organic carbon, to the nitrogen available for nitrification in the wastewater (C:N) is one of the critical factors affecting the nitrification systems design.

Usually, in all systems there is organic matter, which allows the growth of heterotrophic bacteria. In this situation, the growth rate of heterotrophic bacteria is higher than the growth rate of autotrophic bacteria. This domination of heterotrophic bacteria represent a danger because their growth exceeds the nitrifying bacteria growth rate, ultimately leading to the elimination of nitrifying bacteria from the system [12] [13].

In this case, if we want nitrification to occur, we must take into account the following equations in the nitrification systems dimensioning [13]:

$$\mu_{max\ N} > \mu_b \tag{22}$$

where,

$\mu_{max\ N}$ – maximum growth rate of the nitrifying bacteria [day^{-1}];

μ_b –growth rate of the heterotrophic bacteria [day^{-1}].

$$\mu'_N = \mu_{max\ N}\left(\frac{DO}{K_{o2}+DO}\right)(1 - 0.833(7.2 - pH)) \tag{23}$$

where,

μ’N –maximum possible growth rate of nitrifiers under environmental conditions of T, pH, DO and N >> KN[day-1].

$$\mu_N = \mu'_N\left(\frac{N}{K_N+N}\right) \tag{24}$$

A more rigorous condition, if we want nitrification to occur is [13]

$$\mu'_N > \mu_b \tag{25}$$

Thus, in order to build and sustain a sufficient nitrification population, the following condition must be met [4]:

$$\theta_c^d > \theta_c^m \tag{26}$$

where,

θ_c^d – design solids retention time [d]

θ_c^m – minimum solids retention time for nitrification at environmental conditions within the system [d]

The net specific growth rate of the heterotrophic population can be expressed as [4] [13]:

$$\mu'_b = \frac{1}{\theta_c^d} = Y_b q_b - K_d \tag{27}$$

where,

μ'_b – net specific growth rate of heterotrophic bacteria [day^{-1}];

Y_b – true heterotrophic yield coefficient [g VSS grown/g of substrate CBOD (or COD) removed];

q_b – rate of organic removal [g CBOD (or COD) removed/g of active VSS/day];

K_d – decay coefficient [day^{-1}].

The rate of organic removal is defined as [4] [13]:

$$q_b = \frac{S_0 - S_1}{X_1 T} \tag{28}$$

where,

S_0 – influent total CBOD (or COD) [mg/l];

S_1 – effluent soluble CBOD (or COD) [mg/l];

T – system hydraulic retention time [day];

X_1 – system active total VSS [mg/l].

Assuming that both Y_b and K_d are constant, the only way μ'_b or θ_c^d can be manipulated is by changing q_b. One way in which the substrate removal rate can be reduced is by placing an organic carbon reduction step before the nitrification step, creating a "separate step" nitrification process. The result of this procedure is to reduce the food available to heterotrophic bacteria and reduce their dominance in controlling the retention time of solids. Another way (without separating the processes of carbon oxidation and nitrification) is to increase the biological solids in the system, which can be achieved by increasing the concentration of biological solids under aeration or by increasing the volume of the oxidation tank while maintaining biological solids at the same concentration.

3.8. The influence of oxidation-reduction conditions

Usually in the case of domestic wastewater treatment plants, the processes of carbon oxidation, nitrification, denitrification and biological removal of phosphorus in one or more biological reactors are combined [4].

In these systems, nitrifying bacteria are exposed to anoxic and anaerobic conditions. There are studies that have investigated whether there is an impact of reducing or lacking dissolved oxygen concentration on nitrifying bacteria and finding that exposure to anoxic or anaerobic conditions lasting for hours does not have a significant impact on the effectiveness of nitrifying bacteria, if at the end of anoxic cycles or anaerobic, acceptable dissolved oxygen concentrations are restored for nitrification [4].

4. Conclusions

Nitrogen is an essential nutrient for the survival of all living organisms. It is found in the form of nitrogen in the atmosphere but cannot be assimilated by plants until it enters the nitrogen circuit in nature, where it is transformed biologically or physically into assimilable forms.

Ammonium is a form of nitrogen that may generate degradation of water quality if it is present in water supply systems. It can be reduced to nitrogen gas through the biological processes of nitrification and denitrification.

Nitrification is the biological process of oxidizing ammonium to nitrate and is usually performed by autotrophic bacteria Nitrosomonas and Nitrobacter. Nitrosomonas is limited to the oxidation of ammonium to nitrite, and Nitrobacter is limited to the oxidation of nitrite to nitrate.

The nitrification process is much more complex than the simple oxidation of ammonium to nitrate, because it involves various reactions and enzymes that help bacteria grow. Microorganisms grow by combining energy-producing reactions with those of cell synthesis.

The biological process of nitrification or microbial activity is affected by a number of factors such as ammonium concentration in water, contact time between water and biomass, sludge age, biomass wash, pH, temperature, dissolved oxygen concentration and other organic and inorganic inhibitors.

For all these parameters, intervals have been identified at which microbial activity is not inhibited and if these ranges values are taken into account, the nitrification process will work successfully.

In addition to the factors mentioned above, there is another problem related to the combination of ammonium oxidation process with the process of reducing carbon and nitrates, when for several hours the conditions become unfavourable for nitrifying bacteria. There are studies that have shown that nitrification works even when conditions become unfavourable for hours, but for safety microbial activity must be monitored.

The kinetics of the nitrification process is the study of factors with an impact on nitrification, factors that are part of the environmental conditions in which the nitrification process will take place. Knowing the environmental factors, a proper design of biological nitrification systems, will work successfully.

References

[1] Bernhard, A. (2010). The nitrogen cycle: processes, players, and human impact [Abstract]. *Nature Education Knowledge.* 3(10), 25.

[2] Biologydictionary.net Editors. (2021, January 15) *Nitrogen Cycle*. Retrieved December 2, 2021, from https://biologydictionary.net/nitrogen-cycle/

[3] Galloway, J. N., Leach A. M., Bleeker A. & Erisman J. W. (2013) A chronology of human understanding of the nitrogen cycle. *Phil. Trans. R. Soc. B.* 368(1621), 20130120. DOI: 10.1098/rstb.2013.0120

[4] United States Environmental Protection Agency. (1993). *Manual - Nitrogen Control.* Washington DC: Office of Research and Development Office of Water.

[5] Khan Academy. *The nitrogen cycle.* Retrieved December 2, 2021, from https://www.khanacademy.org/science/biology/ecology/biogeochemical-cycles/a/the-nitrogen-cycle

[6] Qinli, W., Yuanyuan Z., Wangxi L., Ruili L., Qihua H., Xiaohong F., Roberto D. M., Cindy A., Nicolaus W. & Jinxing L. (2013). Single-particle analysis reveals shutoff control of the Arabidopsis ammonium transporter AMT1;3 by clustering and internalization. *Proc. Natl. Acad. Sci. USA.* 110(32), DOI: 10.1073/pnas.1301160110.

[7] Radu, G. (2018). *Inventarul surselor de apa cu continut ridicat de amoniu din Romania*. Bucharest: Technical University of Civil Engineering of Bucharest. (No.1)

[8] Painter, H. A. (1970). A review of literature on inorganic nitrogen metabolism in microorganisms. *Proc. Water Res.* 4(6), 393-450. DOI: 10.1016/0043-1354(70)90051-5.

[9] Shah, D. B. & Coulman G. A. (1978) Kinetics of nitrification and denitrification reactions. *Biotechnol. Bioeng.* 20(1), 43-72. DOI: 10.1002/bit.260200105

[10] Calin, C. (2011) *Procese si Tehnologii pentru Controlul Continutului de Azot din Apa*. Unpublished doctoral thesis, Technical University of Civil Engineering of Bucharest.

[11] Radu, G. (2019) *Stadiul actual privind tehnologiile de tratare a apei cu conţinut ridicat de amoniu.* Bucharest: Technical University of Civil Engineering of Bucharest. (No.2)

[12] Halling-Sørensen, B. & Jorgensen S.E. (1993). *Studies in Environmental Science.* Elsevier Science Retrieved November 27, 2021, from Elsevier Science Link https://www.sciencedirect.com/science/article/abs/pii/S0166111608705259. DOI: 10.1016/S0166-1116(08)70525-9

[13] United States Environmental Protection Agency. (1975). *Process Design Manual for Nitrogen Control.* Washington DC: Office of Technology Transfer of USEPA.

[14] United States Environmental Protection Agency. (2010). *Nutrient Control - Design Manual.* Washington DC: Office of Research and Development.

[15] McCarty, P. L. (1965). Thermodynamics of biological synthesis and growth. *Air Water Pollut.* 9(10), 621-39. PMID: 5837726.

[16] Grady, C. P. L. Jr., Daigger G. T., Love N. G. & Filipe C. D. M. (2011). *Biological Wastewater Treatment (third ed.).* USA: IWA Publishing and CRC Press.

[17] Poduska, R. & Andrews J. (1975). Dynamics of nitrification in the activated sludge process. *Water Pollut. Control Fed.* 47(11), 2599-619. PMID: 1219141.