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# METHODS AND APPARATUS OF MICROBIOLOGICAL NITRIFICATION

# METODY I APARATY PROCESU NITRYFIKACJI MIKROBIOLOGICZNEJ

A b s t r a c t

A very important environmental problem around the world is spontaneously or artificially arising pollution of water and sewage. One of such pollutants are nitrogen compounds. Current methods and apparatus used to eliminate or reduce the occurrence of these compounds in water and wastewater are presented in the paper. Also the latest developments in reducing nitrogen pollution are discussed.

*Keywords: bioengineering, nitrification, environment protection*

S t r e s z c z e n i e

Ważnym problemem ekologicznym na całym świecie są powstające samoczynnie lub sztucznie zanieczyszczenia wód i ścieków. Jednym z takich zanieczyszczeń są związki azotu. W pracy przedstawiono używane obecnie metody i aparaty służące do eliminacji, bądź zmniejszenia ilości występowania tych związków w wymienionych źródłach, czyli wodach i ściekach, a także najnowsze osiągnięcia w celu redukcji tych zanieczyszczeń.

*Słowa kluczowe: bioinżynieria, nitryfikacja, ochrona środowiska*

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## 1. Sources of water and wastewater containing ammonium ion

Water is a substance widely available and present in almost all aspects of human life. Its composition is shaped by numerous factors, including the natural phenomena which occur in it and which come from both the soil and rock environment, as well as it depends on the source of water, i.e. on the pollution level of the region of its occurrence. The substances of natural origin are treated as its dopants, all others are regarded as contaminants. Currently, the most polluted is surface water, the least polluted appears to be groundwater. However, the contact of the latter with surface water, as well as with precipitation and anthropogenic pollution, increases the risk of its even higher level of contamination. It is clear, therefore, that the deeper the water occurs and the greater integrity of the environment in its vicinity, the lower the risk of its contamination [1].

Ammonium ions predominantly exist in municipal – industrial and commercial – wastewaters. The presence of these ions in surface waters and groundwaters indicates that there was decomposition of organic substances of plant or animal origin. The occurrence of ammonium ions causes a number of environmental problems, including eutrophication in surface waters, toxic effects on the water fauna, as well as reducing the effectiveness of disinfenction and increased consumption of oxygen dissolved in water [2].

To reduce the risk of contamination, sewage must be subject to treatment, aiming at the reduction of concentrations of compounds adversely affecting the aquatic environment.

## 2. Chemism of nitrification and denitrification as well as microorganisms used

The most commonly applied industrial method which allows for the removal of nitrogen compounds from contaminated water and wastewater is the method of biological nitrification and denitrification, with the participation of relevant bacterial strains. According to the thermodynamic equilibrium of hydrolysis and electrolytic dissociation, ammonia nitrogen in wastewater occurs in two forms: ionized ($NH_4^+$) and unionized ($NH_3$). Both forms are highly toxic to fish from water contaminated with these compounds. Maximum concentration of ammonium in water with fish farming is 0.0125 mg/l (some sources say 0.025 mg/l) [3].

The contribution in concentrations of non-ionized ammonia compounds in comparison with the ionized form depends on the temperature, pH and salinity of aqueous solutions under consideration. Studies show, however, that at low concentrations, $NH_3$ is a more toxic form. The increased occurrence of the non-ionized form is present under conditions of low pH and high temperature [4].

Microbiological removal of nitrogen from sewage water is carried out in two stages: nitrification and denitrification. Nitrification is an aerobic process. During the first stage of nitrification, ammonium nitrogen, as an energy substrate, is oxidized to nitrites. Autotrophic bacteria occurring at this stage of wastewater treatment are mainly *Nitrosomonas*. During the second stage, the resulting nitrites are then oxidized to nitrates by *Nitrobacter* bacteria. In natural environments, during the oxidation of ammonium ion are also present: *Nitrosococcus*, *Nitrosospira* or *Nitrosocystis*, while in the second stage: *Nitrospira* and *Nitrococcus* [5].

The nitrification process (stage I and II) are described by the stoichiometric equations, respectively

$$2NH_4^+ + 3O_2 \rightarrow 4H^+ + 2H_2O + 2NO_2^- \tag{1}$$

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

Biological conversion of the $NO_3^-$ ions to free nitrogen, or denitrification, requires an anaerobic environment. The process is carried out in the presence of electrodonor compounds.Denitrification reaction can be written by the equation (3)

$$2NO_3^- + 12H^+ \rightarrow N_2 + 6H_2O \tag{3}$$

As it can be noticed, an integral part of nitrogen removal process is an intermediate stage of the formation of nitrites. In stage II of the nitrification, 25% of oxygen is consumed, while in stage I – 75%, taking into account the whole process [6].

## 3. Methods and apparatus of nitrification

In recent years, numerous technologies of water and sewage treatment of nitrogen--containing compounds were developed. Also, an apparatus for carrying out these processes is still being improved and new solutions appear. The best known process of wastewater treatment, which has been used for a long time until today is wastewater treatment with activated sludge. However, over time, fluidized bed reactors and biofilters have been introduced in treatment technologies, based on the formation of a biofilm consisting primarily of microorganisms that help to remove toxic compounds. Membrane reactors are innovative solutions, whose appropriate activity is still subject to scientific research. It can be expected that in the future they will constitute the basic equipment in wastewater treatment technology.

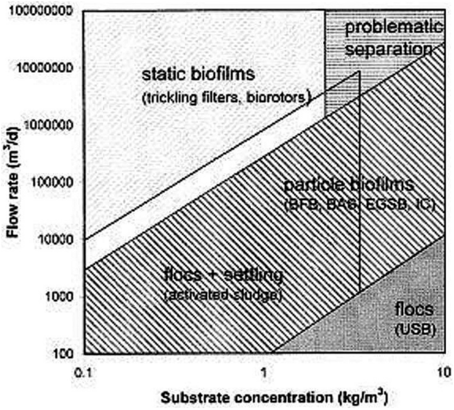


Fig. 1. Phase diagram of sewage flow rate – substrate concentration relation for biofilm and activated sludge reactors

Rys. 1. Diagram fazowy zależności natężenia przepływu ścieków od stężenia substratu dla reaktorów z biofilmem i osadem czynnym

In the microbiological treatment of wastewater from nitrogen compounds, three types of aggregation of microorganisms are used:
1) static biofilm, used for example in the processes occurring in biofilters,
2) molecular biofilm (biofilm on the fine-grained vehicles and granular biofilm), used for example in fluidized bioreactors,
3) flocs, present in treatment which uses activated sludge.

Figure 1 presents a phase diagram showing the use of the above-mentioned forms of aggregation of microorganisms for different types of bioreactors, depending on water flow rate and substrate concentration [7].

### 3.1. Activated sludge

Activated sludge is a group of microorganisms, forming a flocculent suspension consisting of heterotrophic bacteria and possibly protozoa. Among the protozoa in activated sludge, flagellates, amoeboids and ciliates are present, whose role, although peripheral, is valid. Protozoa are an indicator for activated sludge wastewater treatment. A large number of flagellates indicates an overload of the sludge, that is, its expansion, while a large number of ciliates – the proper conditions for activated sludge. In addition, protozoa feed on bacterial cells, thereby forcing the bacteria to multiply, and therefore provide rejuvenation and activation of the sludge. This creates a dual trophic level, which should be reflected in the kinetic models of the process [8].

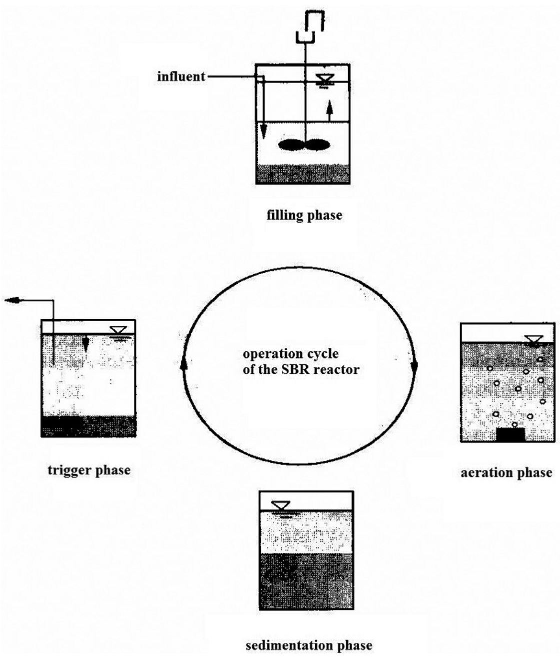


Fig. 2. Operation cycle of SBR reactor [5]

Rys. 2. Cykl działania reaktora SBR

The nitrification process using activated sludge is carried out in reactors operating under flowing or fed-batch conditions, depending on a type of the process. Figure 2 shows a diagram of the fed-batch reactor, called Sequential Batch Reactor (SBR) [5].

Flow reactors used for the nitrification process can operate as a single unit or as a cascade of reactors. They can be divided into:

– reactors with activated sludge chamber with complete mixing,
– plug flow, or dispersion, reactors e.g. circular channel.

In the process of wastewater treatment using activated sludge technology, as shown in Fig. 3, air or oxygen are introduced into the liquid mixture consisting of wastewater and biomass. This mixture will undergo a process of purification, together with organisms, which will form biological flocs. The aim is to reduce the content of organic compounds in wastewater. Such a process is called bioflocculation. After the initial treatment, the mixture is fed to the sedimentation tank, where the purified upper layer of precipitate, the so-called supernatant, is withdrawn from the process. The remaining precipitate is recycled back into the aeration system. This fraction is called "Return Activated Sludge" (RAS). The resultant excessive sludge is called "Waste Activated Sludge" (WAS). It is completely removed from the treatment process, in order to maintain a balance between the amount of biomass and wastewater [9].

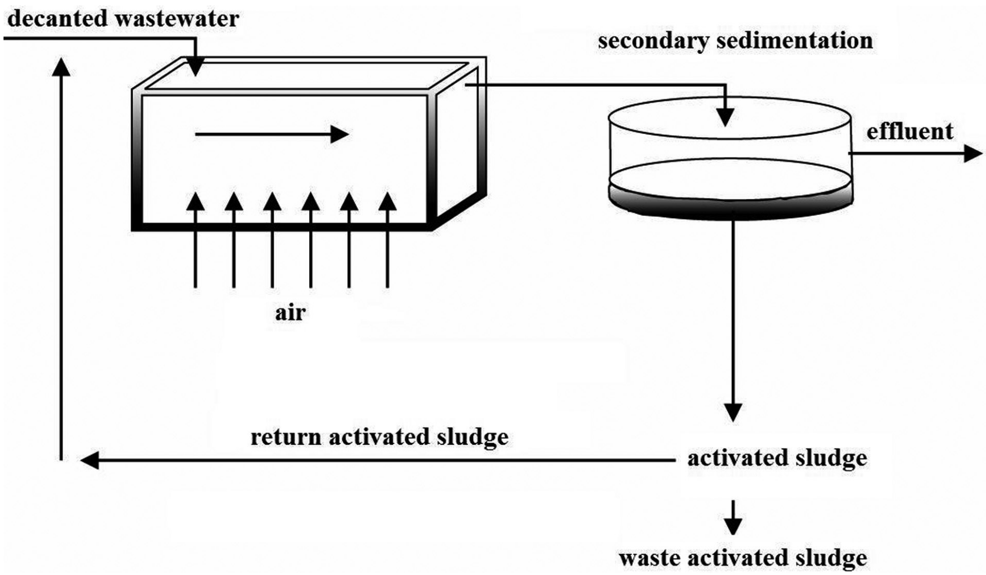


Fig. 3. Simplified scheme of nitrogen compounds removal by activated sludge in wastewater treatment process

Rys. 3. Uproszczony schemat procesu oczyszczania ścieków ze związków azotu za pomocą osadu czynnego

## 3.2. Biofilms

Biofilm is a layer consisting of microorganisms and exopolysaccharides (EPS), immobilized on a surface of a vehicle. EPS are polysaccharides with a clearly defined structure, produced by microorganisms.

Development of the biofilm is accompanied by numerous changes, such as adsorption and desorption of microorganisms to and from the solid surface, attaching to the surface, biofilm growth and its breaking. In the first stage of biofilm formation, the organic or inorganic molecules adsorb on the surface, forming the so-called layer of air conditioning. When this layer is formed, the next step is to increase the adhesion ability of microorganisms to it. This is influenced by various factors, such as pH and temperature of the contact surface, the rate of fluid flow over the surface, the availability of nutrients, the duration of contact of bacteria with the surface, the degree of bacterial growth and surface hydrophobicity [10].

To carry out the process of biofilm nitrification the following bioreactors are used:

– USB (Up-Flow Sludge Blanket); these are devices that use an anaerobic process, which leads to the emergence of a granular sludge layer. The sewage flowing upward through the sludge layer is degraded by anaerobic microorganisms. The microorganisms grow, creating a layer of biofilm in the form of pellets.

– BFB (Biofilm Fluidized Bed) is an innovative technology in wastewater treatment. It is an integrated system which uses both the elements of treatment technology using activated sludge and the growth of microorganisms in the form of a static biofilm.

– EGSB (Expanded Granular Sludge Blanket) is a variant of the USB reactor, using granular sludge. This is a new technology, thanks to the possibility of obtaining high-speed deposition and activity of the biofilm.

– BAS (Biofilm Airlift Suspension); they have an inner recycle stream, thus low residence times for the reactors with a small volume can be achieved. Also in wastewater treatment technologies they are attractive due to the achieved required degree of mixing without the use of a mechanical agitator.

– IC (Internal Circulation); they have two sets of three-phase separation modules that separate gas, liquid, and biomass. It helps in retaining biomass, and thus allows for an increase in its activity, and ultimately improves the quality of sewage [11].

The application of molecular biofilm reactors in the process of nitrification has many advantages, including high concentrations of biomass and a large area of interfacial mass transfer, resulting in the possibility of obtaining a high degree of conversion of substrates [7].

### 3.2.1. Biofilters

Biofilters are defined as reactors with a stationary layer of the bacterial film required for biological oxidation of ammonium ion [12]. In biofilters only active biomass on the filter vehicle is responsible for the oxidation of the substrate, regardless of the total biomass present in the biofilm. The thickness of the biofilm depends upon many factors, including the concentration of the substrates, water flow and temperature. The thickness of the biofilm, resulting from the bacterial growth on the filter affects the nitrification performance of the filter. With excessive growth of microorganisms in the anaerobic biofilm layer, the cells deprived of nutrients and oxygen begin to die. Dead cells reduce the adhesion of the biofilm to the vehicle surface, which ultimately leads to breaking the biofilm. As a result, the ammonia removal efficiency clearly decreases. Dead layers of the biofilm are removed and renewed to ensure continuity of the nitrification process. For this reason, flushing of the biofilter is applied periodically, which is essential for the maintenance of good conditions and the continuity of the process. Between two flushings, an analysis of aquaculture used to convert ammonia to nitrite and nitrite to nitrate is performed [13].

#### 3.2.2. Dual-phase Fluidized Bed Bioreactors with a separate aeration module

As it was mentioned earlier, in the biological treatment of nitrogen sewage, two types of processes involving bacteria are used. Namely, the bacteria can grow as a result of the suspension (e.g. in the activated sludge) or immobilization on the surface of solid vehicles (e.g. in biofilters). The idea of a biological fluidized bed is based on these two processes, because bacteria grow on the surface of small solid particles, resulting from the fluid suspension.

The fluidized bed can operate as a two-phase system: solid – liquid phase, as well as three-phase, additionally taking gas into account. In a two-phase system, an outside oxygenerator plays an important role, which is responsible for oxygenation of the liquid phase. It is located in a loop of the liquid recycle. An important design task is to match the speed of interfacial transfer of oxygen to the reactor size and activity.

Hydrodynamic state of the two-phase fluidized bed is controlled by a fluid flow. For a given solid – liquid system, the bed remains stationary when the fluid speed is lower than the minimum fluidization velocity. When the fluid velocity exceeds the minimum fluidization velocity, the bed turns into the fluidized state. Under these conditions, the solid particles are almost uniformly suspended in a liquid, so they are in good contact with the liquid phase, with perfect interfacial mass transport. With further increase in the fluid velocity, solid grains are starting to be lifted from the bed [14].

#### 3.2.3. Three-phase Fluidized Bed Bioreactors

Three-phase fluidized bed bioreactors used in technologies of wastewater treatment of nitrogen-containing compounds, work in the system: gas – liquid – solid [15]. Biograin constitutes a solid phase, the air is a gas phase, and the purified wastewater is a liquid phase [16].

High performance of fluidized bed bioreactors is achieved by such factors as:

– immobilization of cells on or in grains of solid vehicles, so very high biomass concentrations can be achieved, even exceeding 30-40 kg/$m^3$,

– residence time distribution for the liquid phase and immobilized microbial cells, so that restrictions on the flow rate imposed by the maximum rate of microbial growth were eliminated, as it takes place in flow tank reactors with the activated sludge,

– intensive contact between the solid and liquid phases,

– the use of mobile vehicles allows to gradually supplement the fluidized bed without interrupting the operation of the apparatus, which ensures the maintenance of high microbial activity [17].

An example of the three-phase bioreactor is the *airlift* apparatus shown in Figure 4. The typical *airlift* reactor consists of two zones: the ascent and descent zones. Inert material such as sand, coal or ceramic materials can be used as vehicles in the reactor, on the surface of which immobilization and growth of microorganisms occur. Such reactors provide intensive oxygen transport, mixing and relatively even distribution of the vehicles [18].

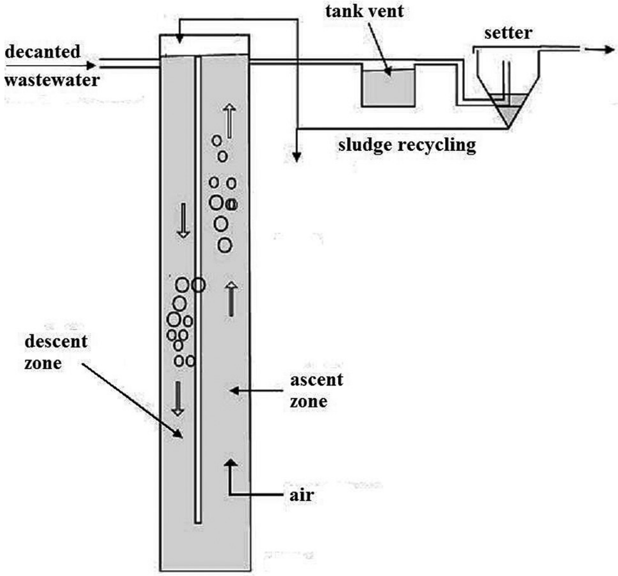


Fig. 4. Scheme of a three-phase airlift bioreactor

Rys. 4. Schemat trójfazowego bioreaktora airlift

## 4. Bioreactors and membrane processes

The growing interest in membrane reactors is due to the simplicity of their construction and the fact that they allow easy operation of continuous processes, which facilitates the conduct of, usually reversible, reactions toward the products. The removal of ammonium ion from wastewater is carried out, inter alia, in the Membrane Biofilm Fiber Reactor. MBFR can be described as a system of hollow fibres supplying gas to the biofilm which grows on the outside of these fibres [19].

Membrane reactors used in wastewater treatment technologies are used particularly in the aerobic treatment of municipal wastewater. Membrane systems are characterized by numerous advantages, including: high level of biomass in the reactor, the reduction of its volume and the improvement of product quality. The membranes do not just retain the whole biomass, but they also prevent the escape of extracellular enzymes. In practice, wastewater treatment technologies commonly use membranes with pore sizes from 0.1 to 0.4 microns, i.e. those which correspond to the microfiltration process. In membrane reactors, purification of soluble compounds of high molecular weight is also carried out.

The disadvantage of membrane reactors is their high cost, resulting from high prices of membrane systems as well as high energy costs, resulting from the necessity to maintain an adequate pressure gradient. In addition, inappropriate selection of a membrane can cause unfavourable changes in the activity of microorganisms. This is due to the retention of metabolic products in the bioreactor, which tend to hinder life processes of microorganisms [20].

## 5. Development prospects of research on the biological nitrification process

Currently, a great deal of research aimed at improving the well-known methods of wastewater treatment is being conducted. This is done by modifying the existing treatment systems into smaller, more efficient and more economic ones, due to the cost of entire systems and processes. Research and development of nitrification technology is most often carried out on a laboratory scale. There is, as yet, insufficient information on the modelling of membrane processes, anaerobic processes and pelletization of biomass.

One of the problems of wastewater treatment technology is to achieve low levels of ammonia concentration (below 2 $gN/m^3$) in wastewater from sewage treatment with activated sludge. To achieve this goal, a thorough analysis of the process in terms of the kinetics of wastewater decomposition under different conditions of oxidation, microbial activity and the conditions of mixing in the aeration tank will probably be required.

Economy of the nitrification process could be improved if the process was completed with the formation of nitrites. This is associated with a lower use of oxygen, as well as more effective denitrification of nitrites. Many studies have shown that the nitrification process can be inhibited at the stage of formation of nitrites with nitric acid and non-ionized ammonia. *Nitrosomonas* and other bacteria (so called nitrosobacteria) of the first stage of the nitrification, responsible for the oxidation of the ammonium ion, produce nitric acid. This acid strongly inhibits the oxidation reaction, in which it is *de facto* formed and is secreted outside the cell. In turn, the oxidation of nitrites in the second stage of the nitrification is inhibited by the ammonium ion. Then incomplete nitrification takes place, resulting in the accumulation of nitrites in the environment [21].

A new method of removing nitrogen compounds from wastewater and water is the *Anammox* process. It is a process of anaerobic ammonia oxidation carried out by bacteria. Removal of nitrogen without the use of dissolved oxygen is the biggest advantage of this process. Definitely the attractiveness of this process is supported by the fact that the microorganisms that carry out this process do not require an addition of any organic compound which is necessary for denitrifying bacteria. In short, this process can be represented by the following reaction

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \tag{4}$$

The intermediate products of the process are hydrazine and nitrogen oxide (II). At the moment the application of the process is scanty. This is due to the novelty of the process on the one hand, and on the other to a very slow pace of growth of the bacteria used in this process. This means that keeping in the device running on an industrial scale an adequate amount of bacteria to carry out the process requires a very long life of the biomass. However, the economy of the process, which is understood as the reduction in aeration power, no need for dosage of external carbon sources and the emergence of minimal amounts of the biomass, make this process very popular. Therefore, more and more treatment plants opt for such a solution.

Recently, a competitive method of removing nitrogen from wastewater has appeared, namely the cathode reduction of nitrates in the Microbial Fuel Cell. A novelty is that both carbon and nitrogen can be removed in this process. MFC is a new technology based on the fact that the energy contained in the organic matter is directly converted into useful electricity.

Power generation is the result of metabolic processes of microorganisms. In the Microbial Fuel Cell the bacteria attach to a metal electrode and transmit electrons to it. In order for such a cell to be able to function, the bacteria must be supplied with the right kind of organic waste as a culture medium [22].

## 6. Conclusions

Ammonium ion is considered to be one of the most important parameters in monitoring and assessing water quality. Knowledge of the issues relating to the conduct of a process aiming at its elimination, or microbial nitrification and denitrification, is a key task in wastewater treatment technology. The paper presents a description of this process, wastewater treatment technologies used so far, and the equipment serving these purposes. It has been proved that the issues related to the removal of nitrogen-containing compounds during the process of water and wastewater treatment in the form of the ammonium ion and nitrites are an important element in environmental protection. Methods of reducing nitrogen compounds, which are beginning to become competitive to the previously used ones, have been discussed. It is estimated that full understanding and mastery of the nitrification process alone will require many more years of research. Therefore, numerous innovative technological and equipment solutions associated with this process can be expected.

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