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## ANAEROBIC CONVERSIONS OF AMMONIUM NITROGEN IN AQUATIC ECOSYSTEMS

Conversions of nitrogen through Anammox processes in aquatic ecosystems can occur in deeper levels of water and in bottom sediments, participating significantly in the full cycle of nitrogen circulation. This paper shows the present condition of knowledge in this field, based on the analysis of the available literature.

### 1. INTRODUCTION

In aquatic ecosystems, nitrogen can be found in various forms, i.e. gaseous (mainly  $N_2$ ) and dissolved forms or in organic or inorganic combinations. It is one of biogenic elements whose excess in water bodies may distort the balance and intensify the process of biomass creation, contributing in this way to the acceleration of water eutrophication. In aquatic environment, it undergoes constant biochemical conversions, both in the water column and in bottom sediments. Bottom sediments play a crucial role in nitrogen circulation in aquatic ecosystems, and the substances they contain may become one of the basic factors influencing the quality of water [7], [12], [19], [32].

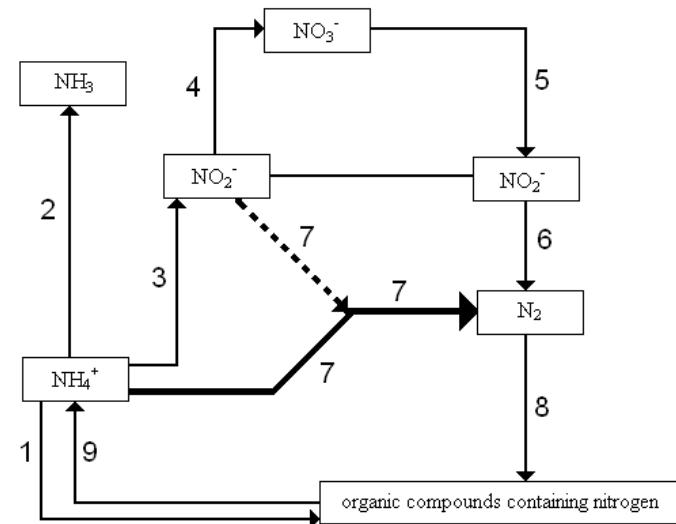
### 2. CONVERSIONS OF NITROGEN COMPOUNDS IN AQUATIC ENVIRONMENT

Transformations of nitrogen compounds in supernatant water–bottom sediments interphase boil down mainly to the processes of nitrogen bonding, ammonification, nitrification, assimilation of nitrates and dissimilatory reduction of nitrates (denitrification and reduction to ammonium nitrogen) (the figure) [19], [30], [32]. These proc-

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esses were extensively researched, hence they are well known. However, careful observation of the above-mentioned processes allowed other mechanisms of nitrogen conversions in aquatic environment to be specified [32].



Nitrogen conversions occurring in aquatic ecosystems

(1 – biomass synthesis, 2 – stripping, 3 – partial nitrification [ $\text{NH}_4^+$  to  $\text{NO}_2^-$ ],  
 4 – partial nitration [ $\text{NO}_2^-$  to  $\text{NO}_3^-$ ], 3 + 4 – nitrification, 5 – partial denitrification [ $\text{NO}_3^-$  to  $\text{NO}_2^-$ ],  
 6 – partial denitrification [ $\text{NO}_2^-$  to  $\text{N}_2$ ], 5 + 6 – denitrification, 7 – Anammox, 8 – assimilation,  
 9 – ammonification) [7], [9], [19], [21], [30], [34], [38]

### 3. ANAEROBIC OXIDATION OF AMMONIUM NITROGEN (ANAMMOX) IN AQUATIC ECOSYSTEMS

Detection of the presence of new microorganisms participating in sewage treatment with the method of activated sludge resulted in partial reconstruction of the classical nitrogen cycle through the separation of the indirect channel (figure 1) [1], [5], [10], [18], [32], [33], [35]. The process described as Anammox (**A**naerobic **A**mmonia **O**xidation) consists in direct oxidation of ammonium nitrogen to molecular nitrogen in anaerobic conditions [1], [2], [10], [21], [32]. This oxidation is performed by autotrophic microorganisms using nitrites as electron acceptors [2], [10], [33]. In sewage treatment, Anammox can be carried out in one- or two-reactor systems with activated sludge, with biofilm or in hybrid and membrane systems, both in flow and portion channels [1], [16], [18], [32], [33], [35], [39].

Three types of bacteria, i.e. *Brocadia* (*B. anammoxidans* and *B. fulgida*), *Kuenenia* (*K. stuttgartiensis*), *Scalindua* (*S. wagneri*, *S. brodae* and *S. sorokinii*), are able

to carry out the Anammox process [1], [2], [9], [10], [20], [21], [32], [39]. In the *Scalindua* strain only, sea strains were found, while the other ones being separated from the sewage treatment systems [5]. Based on phylogenetic analyses, the bacteria capable of carrying out the Anammox process belong to monophyletic microorganisms and are classified as *Planctomyces* [2], [20], [21], [30], [32], [38], [39].

In order to determine and quantify *anammox* bacteria in aquatic ecosystems, various combinations of microbiological and biogeochemical techniques are used: 16S rRNA gene sequencing, FISH technique (fluorescence in situ hybridization), tests with labelled nitrogen  $^{15}\text{N}$ , membrane lipid analysis, nutrient profiles, and enrichment cultures [9], [20].

Little is known about the growth rate of *anammox* bacteria in aquatic ecosystems. Taking into account the fact that these bacteria are related to those present in sewage treatment plants and growing very slowly (up to 9 days under adverse conditions [29] and at optimal temperature of 37 °C [13]), what may be anticipated is a slower growth of bacteria living at much lower temperatures that are present in aquatic environment, especially in seas. However, the optimal temperature for the growth of the aquatic populations of *anammox* bacteria seems to adjust to a local environment. Research under ex situ conditions showed that in permanently cold deposits of Young Strait (Greenland) whose average annual temperature is below 1 °C, the optimal temperature for *anammox* bacteria was 12 °C, and in Skagerrak (Denmark), whose average annual water temperature is between 4 and 6 °C, the optimal temperature was 15 °C [5], [6], [24]. The optimal pH ranges from 5.2 to 7.1. Above and below this range the activity of the *anammox* bacteria decreases [26], [27]. The efficiency of the Anammox process depends on the growth of the bacteria [11] that require stable conditions and long development time [5].

TRIMMER et al. [34] arrive at the conclusion that the activity of the *anammox* bacteria in the sediments depends on the availability of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , because the enzymes of these bacteria are active under conditions of constant feeding with  $\text{NO}_x^-$  [20]. The availability of  $\text{NO}_2^-$  probably controls the number and activity of *anammox* bacteria and is strictly related to the activity of other groups of bacteria due to the production of  $\text{NO}_2^-$  as a semi-product in the processes of nitrification, denitrification and dissimilatory reduction of nitrates [20], [21]. ENGSTRÖM et al. [8] discovered the dependence between  $\text{N}_2$  produced as a result of the Anammox process and the depth of water, mineralized dissolved substance, consumption of oxygen by benthos and the amount of chlorophyll on the surface of sediments. These correlations suggest fierce competition between various reducers of nitrates in water [20], [21]. For instance, the Anammox process was responsible for the production of 67.24 and 2%  $\text{N}_2$  in the sediments of Skagerrak (Denmark) at the respective depths of 700 and 380 m. Due to this, the Anammox process plays a greater role in the deeper levels rather than in shallow layers of the bodies of water [5].

The first publication about the Anammox process in aquatic ecosystems was published in 2002 by THAMDRUP and DALSGAARD [31]. The authors used the technique

with the labelled  $^{15}\text{N}$  to discover and to determine the efficiency of the Anammox process in the sediments collected at a depth of 695 m in Skagerrak (Denmark). The results showed that the Anammox process may be responsible for 67% of the  $\text{N}_2$  produced [8], [21], [31].

Table

Distribution of *anammox* bacteria in natural aquatic ecosystems

Place	Method of detection	Reference
Skagerrak (North Sea)	$^{15}\text{N}$ , nutrient profiles	[5], [6], [8], [20], [21], [31]
Black Sea	$^{15}\text{N}$ , nutrient profiles, FISH, lipids, clone library	[5], [8]–[10], [14], [20], [21], [28], [36], [37]
Golfo Dulce (Costa Rica, Pacific Ocean)	$^{15}\text{N}$ , nutrient profiles	[4], [5], [8]–[10], [20], [36]
Thames Estuary (United Kingdom)	$^{15}\text{N}$	[20], [34]
Arctic Sea (Greenland)	$^{15}\text{N}$ , nutrient profiles	[20], [23]
Mertz Sea (Antarctica)	clone library	[20]
Randers Fjord (Denmark)	$^{15}\text{N}$ , nutrient profiles, FISH, clone library	[5], [20]–[22]
Benguela (Namibia)	$^{15}\text{N}$ , nutrient profiles, FISH, lipids, clone library	[20], [21]
Chesapeake Bay (U.S.A.)	$^{15}\text{N}$ , FISH, clone library	[20]
Gullmarsfjorden (Sweden)	$^{15}\text{N}$ , nutrient profiles	[8], [20]
Long Island (U.S.A.)	$^{15}\text{N}$ , nutrient profiles	[5], [8], [20]
Lake Tanganyika (south-east Africa)	$^{15}\text{N}$ , nutrient profiles, lipids, FISH, clone library	[25]

RISGAARD et al. estimated that the Anammox process was responsible for 4–26% of the  $\text{N}_2$  produced in the sediments of Randers Fiord [22]. In the sediments of the continental shelf in eastern and western Greenland, the Anammox process was relatively significant – within the range from 1 up to 35% of the  $\text{N}_2$  produced [5], [24]. The Anammox process is also essential in the production of  $\text{N}_2$  in oxygen-free layers of water. In Golfo Dulce (Costa Rica),  $\text{N}_2$  created during the Anammox process was estimated at 19–35% [4], [5], [36], and in the waters of the Black Sea it may reach from 20 up to 40% [9]. An effective Anammox process was also discovered in arctic sea glaciers. The process in question was not found in the glaciers that were less than a year old, but in single layers of a few-year old ice floes, and it was responsible for over 40% of the  $\text{N}_2$  produced [5], [23].

Little is known about the Anammox process in freshwater ecosystems. The basis for this is the knowledge on the Anammox process in marine ecosystems. It can be anticipated that the Anammox process will be more important in deep, big, oligotrophic lakes [3]. The Anammox process takes place in the freshwater bodies of Lake Tanganyika (Africa), where it accounts for 7–13% of the  $\text{N}_2$  produced [3], [25]. It is

supposed that in Lake Haringvliet (Holland), the Anammox process may be responsible for 36% consumption of  $\text{NO}_3^-$ [15].

#### 4. SUMMARY

Anaerobic oxidation of nitrogen compounds occurring in deeper levels of water and in bottom sediments may significantly contribute to nitrogen conversions in aquatic ecosystems. Detection of the Anammox process may mean that denitrification will no longer be tantamount to getting rid of fixed nitrogen from aquatic ecosystems. That is why it is crucial to carry out further research of the Anammox process in aquatic ecosystems, especially in freshwater ecosystems (lakes, reservoir – dams).

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ANAEROBOWE PRZEMIANY AZOTU AMONOWEGO  
W EKOSYSTEMACH WODNYCH

Przemiany azotu w procesie Anammox w ekosystemach wodnych mogą przebiegać w głębszych warstwach wód oraz w osadach dennych, mając istotny udział w pełnym cyklu krążenia azotu. Przedstawiono obecny stan wiedzy na ten temat, dokonując analizy dostępnej literatury.