Potential Changes on Anammox Activity After Chicxulub Asteroid Impact

Noel Perez, Osmel Martin and Rolando Cárdenas

Abstract The activity of anammox bacteria (AA) can be estimated based on the production of N_2. Known the mechanism of the catabolic reaction, three differential equations are established according to the most important substrates (NO_3^- and NH_4^+) and the pH. The solution of this system might provide a rough estimate of the activity of the anammox, since neither inhibitory effects nor efficiency of the microorganisms is considered. Anammox can be inhibited by ocean acidification, generated by the deposition of H_2SO_4 and HNO_3 during asteroid impact events such as Chicxulub. The magnitude of the inhibition depends on the amount and speed with which the H_2SO_4 is added fundamentally.

Keywords Anammox activity · Asteroid impact · Ocean acidification

1 Introduction

The process of anoxic oxidation of ammonium (anammox) is one of the main processes of biological conversion of nitrogen in nature. It is characterized by the reaction of nitrite with ammonium in anoxic condition to form dinitrogen gas. The study of the anammox process began at the end of the twentieth century. The existence of this process in itself was a great surprise because it was discovered more than one hundred years after the other basic players in the nitrogen cycle, such as nitrogen fixation, denitrification, and nitrification, had been identified. The biological oxidation of ammonium under oxic conditions is already cumbersome; in addition, the harsh conditions required for the chemical oxidation of ammonium,

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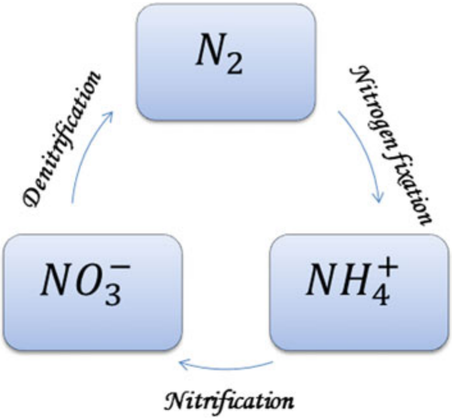
very well known in industrial processes, seemed to be good reasons for the anammox process not to take place.

In 1977, Engelbert Broda predicted that the oxidation of ammonium under anoxic conditions with nitrite or nitrate as an electron acceptor "should exist or exist." Oxidation of ammonium with an electron acceptor other than oxygen was also previously predicted by marine environment researchers, based first on mass balance studies [1], later in combination with a thermodynamic justification [2]. These predictions were presented at a time when it was generally believed that the biological oxidation of ammonium without the presence of oxygen was simply impossible [3].

In 1985, the elimination of ammonium under anoxic conditions was first observed in a pilot-scale denitrification reactor at the yeast factory of the Gist-Brocades bakery in Delf, the Netherlands [4–7]. To achieve the oxidation of sulfur coupled to the reduction of nitrate, nitrate was added and, surprisingly, the elimination of ammonium in this reactor was also produced. This new biological process was called the anammox process. After the initial observations in Delf, in Germany [8, 9] and Switzerland [10], the production of dinitrogen gas instead of nitrate (nitrogen losses) in treated wastewater (rich in ammonia) from leachate was reported of landfills.

Since the second half of the nineteenth century, the biological conversion of nitrogen has had a marked scientific interest. The combination of the reactions of nitrogen fixation (conversion of dinitrogen to ammonium), nitrification (oxidation of ammonium to nitrate), and denitrification (reduction of nitrate to gaseous dinitrogen) allowed to interpret the conversions of nitrogen in nature as a biological cycle (Fig. 1). Based on the above, the cycle was formed that expressed the general view on nitrogen conversions during most of the twentieth century [3].

Fig. 1 Classical nitrogen cycle during twentieth century



2 Conventional Nitrification–Denitrification Processes

2.1 Nitrification

Nitrification is aerobic chemoautotrophic oxidation of inorganic nitrogenous compounds, such as (NH_4^+), hydroxylamine (NH_2OH), and nitrite (NO_2^-) to nitrate (NO_3^-) [11]. Complete nitrification takes place in two stages: the first being the oxidation of NH_4^+ to NO_2^-, and the second the nitrite becomes NO_3^-. Each conversion is carried out by different genera of bacteria. Oxidation of ammonia is carried out by Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosovibrio, and Nitrosobolus [11]. Such bacteria are also called ammonia-oxidizing bacteria, with hydroxylamine as an intermediate reaction [12]. Considering a cellular yield of 0.15 g cells/g NH_4^+–N, the equation for the oxidation of ammonia by Nitrosomonas [13] is as follows:

$$\begin{aligned} & NH_4^+ + 1.3818O_2 + 0.0909HCO_3^- \\ & \quad \rightarrow 0.0182C_5H_7NO_2 + 0.9818NO_2^- + 1.0364H_2O + 1.89H_2CO_3 \end{aligned} \tag{1}$$

The oxidation of nitrite to nitrate is carried out by a diverse group of proteobacteria called nitrite-oxidizing bacteria, among which are Nitrospira, Nitrospina, Nitrosococcus, and Nitrocystis, although Nitrobacter is the most recognized genus [11]. For a cellular yield of 0.02 g cells/g NO_2^--N, the metabolism of nitrite in Nitrobacter follows the reaction:

$$\begin{aligned} & NO_2^- + 0.0025NH_4^+ + 0.01HCO_3^- + 0.01H^+ + 0.048750O_2 \\ & \quad \rightarrow 0.0025C_5H_7NO_2 + 0.0075H_2O + NO_3^- \end{aligned} \tag{2}$$

By addition of the two processes including cell synthesis, the overall equation is represented as follows:

$$\begin{aligned} & NH_4^+ + 1.83O_2 + 1.98HCO_3^- \\ & \quad \rightarrow 0.021C_5H_7NO_3 + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \end{aligned} \tag{3}$$

Nitrification is pH dependent, with the activity decreasing below pH 7.0, and it also consumes alkalinity, which is why it is commonly supplemented with $CaCO_3$ to avoid inhibition by pH [14]. The typical time of retention of sludge in activated sludge systems is between 10 and 20 days [14], due to the low rate of growth of microorganisms.

2.2 Denitrification

Denitrification is the biological reduction of NO_3^- and NO_2^- to N_2 gas. This is usually carried out along a heterotrophic process under anoxic–anaerobic conditions [11]. The complete reduction implies successive reductions with NO_2^-, nitric oxide (NO), and nitrous oxide (N_2O) as intermediates and can be performed by a single type of bacteria. To obtain energy, the different Gram-negative proteobacteria can use a wide range of organic compounds as electron donors and carbon source, as well as nitrates and nitrites as electron acceptors, producing nitrogen gas as the main product. Denitrifying bacteria occupy very diverse niches due to the great metabolic diversity existing between them. Assuming a cellular yield of 0.45 g cells/g NO_3^--N, with methanol as an electron donor [13], the alkalinity is generated in the process as shown in the following equation:

$$\begin{aligned} &NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \\ &\quad \rightarrow 0.056C_5H_7NO_2 + 0.47N_2 + 1.68H_2O + HCO_3^- \end{aligned} \tag{4}$$

Some examples of heterotrophic denitrifiers are: *Pseudomonas, Alcaligenes, Paracoccus, and Thiobacilus.*

2.3 Anaerobic Ammonium Oxidation

The anaerobic oxidation of NH_4^+ (anammox) with NO_2^- as terminal electron acceptor (catabolic reaction), yielding N_2 and NO_3^- as main products, is the recently discovered missing link in the N-cycle [15]:

$$NH_4^+ + NO_3^- \rightarrow N_2 + 2H_2O \quad \Delta G = -359\,kJ\Delta(mol\ NH_4^+)^{-1} \tag{5}$$

Several chemoautotrophic bacteria related to the genus planctomycetes carry out this process. Fundamentally, five genera "Candidatus," Brocadia [16], Kuenenia [17], Scalindua [18], Anammoxoglobus [19], and Jettenia [20] have been studied. Anammox bacteria are found in diverse habitats among them may be mentioned: marine sediments [21, 22], freshwater ecosystems [23, 24], and wastewater treatment plants [7]. These bacteria are considered responsible for up to 50% of oceanic *N* losses [25].

The growth rate of anammox bacteria is low oscillating the doubling time between 11 and 30 days [26, 27]. However, some specific studies have reported duplication times as low as 1.8 days [28] and 3 days [29]. The existence of low growth rates leads to low cellular performance as evidenced by fallow stoichiometry [27]:

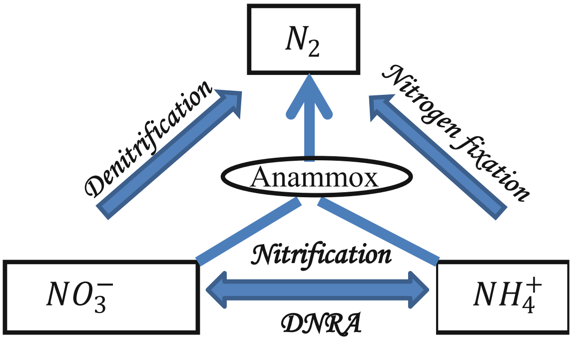


Fig. 2 Current microbial nitrogen cycle (DNRA: Dissimilatory nitrate reduction to ammonium)

$$\begin{aligned} & NH_4^+ + 1.32NO_2^- + 0.066HCO^- + 0.13H^+ \\ & \quad \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03N_2O \end{aligned} \tag{6}$$

The anammox process is used in an emergent way in the treatment of *N*-rich wastewater, especially in those where there is a low *C*/*N* ratio [30], mainly due to the high specific activity of around 0.8 kg N kg of dry weight $^{-1}$ day^{-1}.

Figure 2 shows a more complete picture of the nitrogen cycle in nature after the discovery of the anammox bacteria [30].

3 Kinetic of Anammox Catabolism

In the absence of molecular oxygen, it is very difficult to activate ammonium. For a long time; since the discovery of anammox bacteria, researchers have asked how is it possible that these microorganisms can oxidize ammonium along with the reduction of nitrite to form an N–N bond and produce nitrogen gas. Based on the in silico analysis of the genome assembly of the anammox bacterium Kuenenia stuttgartiensis, a set of three redox reactions (Eqs. 7–9) involving hydrazine (N_2H_4) and nitric oxide (NO) were proposed as intermediates to explain the stoichiometry of global anammox (Eq. 5) [15, 31]:

$$NO_2^- + 2H^+ + e^- \rightarrow NO + H_2O \quad k_1 \tag{7}$$

$$NO + NH_4^+ + 2H^+ + 3e^- \rightarrow N_2H_4 + H_2O \quad k_2 \tag{8}$$

$$N_2H_4 \rightarrow N_2 + 4H^+ + 4e^- \quad k_3 \tag{9}$$

In the above equations, k_1, k_2 and k_3 are the velocities' constants. Using formal kinetics, it is possible to obtain a system of three equations for the reaction rates of each stage that integrate the process described by Eq. (5), as shown follows:

$$\frac{\mathrm{d}[\mathrm{NO}]}{\mathrm{d}t} = k_1[\mathrm{NO}_2^-][\mathrm{H}^+]^2[e] - k_2[\mathrm{NO}][\mathrm{NH}_4^+][\mathrm{H}^+]^2[e]^3 \tag{10}$$

$$\frac{\mathrm{d}[\mathrm{N}_2\mathrm{H}_4]}{\mathrm{d}t} = k_2[\mathrm{NO}][\mathrm{NH}_4^+][\mathrm{H}^+]^2[e]^3 - k_3[\mathrm{N}_2\mathrm{H}_4] \tag{11}$$

$$\frac{\mathrm{d}[\mathrm{N}_2]}{\mathrm{d}t} = k_3[\mathrm{N}_2\mathrm{H}_4] \tag{12}$$

By solving the above system of equations, it is possible to roughly estimate the amount (concentration) of dinitrogen formed during the metabolism of anammox bacteria in any time. Of course, for a more realistic solution, it necessary to consider the inhibitory effect of some subtracts (nitrite and ammonium) or other species presents (hydroxylamine, hydrogen sulfur, dioxygen) and pH of medium, and introducing a term that represents the efficiency of the species. Considering the hypothesis of the steady state could simplify the problem but perhaps run the risk of being a bit unreal. Either way, these are lines of work for the future, which could be extended to other chemosynthetic autotrophs, as, for example, those that live in hydrothermal vents at great depths in the ocean that take advantage of hydrogen sulfide as a substrate.

4 Anammox and Chicxulub Impact

In the Black Sea and in general in all modern anoxic basins, ammonium is the only form of *N*-nutrients in the deep water below the chemocline, which diffuses upwards from the anoxic zone and is effectively consumed by the anammox bacteria before reaching the oxic zone [18]. The anammox bacteria are able to produce a loss of around 40% of the fixed nitrogen that sinks in the anoxic water of this sea. A significant increase in the areal extent of true anoxic conditions in the ocean, most likely could result in further extensive loss of N-nutrients through the anaerobic oxidation of ammonium during the Cretaceous oceanic anoxic event [32].

Most of the paleo-episodes of ocean acidification were too slow or too small as to be instructive in predicting impacts in the near future. The end-Cretaceous event (66 Mya) is intriguing in this regard, both because of its rapid onset and also because many pelagic calcifying species (including 100% of ammonites and more than 90% of calcareous nannoplankton and foraminifera) went extinct at this time. Sulfuric acid addition could have made the surface ocean extremely undersaturated for calcite, but only if they reached the ocean very rapidly (over few days) and if the quantity added was at the top end of the literature estimates [33, 34].

The impact of the Chicxulub asteroid produced at the end of the Cretaceous induced very sudden changes in atmospheric composition, climatic and planetary biodiversity. There was an abrupt and sudden acidification event fundamentally concentrated in the surface waters since the deep waters undergo a delayed and less severe acidification in response to an atmospheric source of acidity. Paleolithic records with which to restrict the changes produced in water chemistry of sea during those few post impact critical years after impact do not exist, and the low rate of accumulation of ocean sediments limit the resolution of sediments records to thousands of years. Tyrrell et al. in their work “Severity of ocean acidification following the end-Cretaceous asteroid impact” used models to calculate how dramatic the acidification of the surface ocean could have been at the end of the Cretaceous [33, 34].

Life on Earth and most likely the biospheres in exoplanets are seriously threatened by the impacts of asteroids and comets. Mass extinction in the Cretaceous–Paleogene (K/Pg) boundary where dinosaurs practically disappeared and approximately 50% of the living genera, it widely accepts as the main contributor the impact of the Chicxulub asteroid. Because of this natural catastrophe, there were several environmental stresses. The most accepted scenario immediately after the impact is “cold and dark.” The aerosols (mainly sulfate aerosols), soot, and dust in the atmosphere completely covered the sunlight at least during half a year (some researchers estimate approximately two and a half years), with the consequent collapse of photosynthesis and global deforestation. The ozone layer was completely destroyed, due to the release of large amounts of chlorine and bromine that form the evaporation of both the asteroid and the target rocks [35]. The target rocks formed mainly by sediments rich in carbonates and gypsum/anhydrite with a granite crust were partially ejected and volatilized as a result of the strong impact. In addition to the sulfur generated by target rock, about $1–5 \times 10^{15}$ mol came from the asteroid itself. The thermal decomposition of gypsum or anhydrite, on the basis of the experiments of volatilization carried out in laboratories, could lead to the almost instantaneous release of sulfur trioxide (SO_3) into the atmosphere according to the reaction:

$$CaSO_4 \rightarrow CaO + SO_3 \tag{13}$$

Once the sulfur trioxide was injected into the atmosphere, it could have been transformed into sulfuric acid by reacting with the water, which would later fall on the ocean in the form of acid rain. Several atmospheric modeling of the K/Pg limit [36, 37] used atmospheric residence times of sulfur from several months to a few years. However, recent studies have proposed an alternative scenario and very different from the one generally accepted for the K/Pg event in which it is suggested [38] that, immediately after the impact, most of the sulfate aerosols (sulfuric acid aerosols) could have been eliminated by the large particles of silicates that fell rapidly back to Earth, delivering the load of H_2SO_4 to the ocean in only one or a few days. Ocean acidification may have affected the metabolism of anammox bacteria, probably leading to inhibition, which should be reflected in their activity. Tyrrell et al. [33] considered additions of sulfuric acid of 15, 30, and 60×10^{15} mol, corresponding to 480, 960, and 1920 Pg, with e-folding timescale from 10 h to 5 years to have a range of possibilities more large. The addition of sulfuric acid reduces the total alkalinity (TA) of the ocean surface water in a molar ratio H_2SO_4 : TA = 1 : 2 [39].

Similar to the production of sulfur compounds due to the vaporization of gypsum rocks, the vaporization of carbonate rocks produces CO_2. The Chicxulub asteroid with approximately a diameter of 10 km hits a layer of sedimentary carbonates of about 3–4 km thick in the Yucatan peninsula [40] releasing between 5000 and 9000 Pg of CO_2 [41] (equivalent to 1300 and 2500 Pg of carbon, respectively). This calculation can be overestimated because possibly the largest amount of carbon dioxide released was greatly reduced due to the rapid recombination [42] of volatilized CaO (around 40–80%) and CO_2 within the impact plume to form calcium carbonate again [43]. After the impact, all the woody biomass could have ignited due to a global thermal shock, which caused forest fires [44, 45] and therefore release large amounts of CO_2 into the atmosphere. It is unlikely that the combustion of carbon from terrestrial vegetation could have contributed much more than 1500 Pg of carbon, both by the aridity of the mid-continent in warmer climates and by the finite habitat space [33]. On the other hand, another potential source of carbon comes from soils. Because of the impact, large amounts of soot, dust, and aerosols were released into the atmosphere, bringing a long period of darkness on the surface of the Earth, during which the process of photosynthesis was strongly inhibited due to low levels of light available [46]. The decomposition of organic carbon (with a rotation time currently of approximately 50 years) [47] could not be balanced with the replacement of the production of leaf litter and other carbon-rich material by living plants. Tyrrell et al. assumed a maximum total for the Upper Cretaceous of 2500 Pg C (1600 Pg at present) considering that the carbon reserves of the soil in the Earth at present are much higher toward the poles, particularly in the permafrost regions. Although the Earth was warmer in the late Cretaceous, the lack of ice in Antarctica could have allowed the accumulation of large carbon reserves in the soil [48]. Tyrrell et al. modeled the effect on the oceanic carbonate chemistry of all these combined sources considering carbon additions of 2000, 4000, and 6500 Pg of C. The volatilization of the carbonate rock in the impact region plus the forest fires and the decay of the soil carbon were fast and slow sources of carbon release, respectively. According to the study by Tyrrell et al. [33], large impacts are produced but not as severe as from large sulfuric additions. In contrast to H_2SO_4, both slower and faster additions of CO_2 to the atmosphere cause similar responses in the ocean, because the slow air–sea exchanges of CO_2 delay the onset of ocean acidity.

The asteroid (and the subsequent ejection) when crossing the atmosphere did so at high speed and the intense wave pressure associated with it should have favored the formation of NO_x from the atmospheric N_2 and O_2. When NO_x reacts with water present in the atmosphere, it forms nitric acid (HNO_3) which, when incorporated into the rain, can induce ocean acidification (similar to what occurs with SO_3) in the following months or years (or possibly days if it is scavenged by the large silicate particles). The total amount of HNO_3 is generated mainly by three causes: direct production of the initial pressure wave (1×10^{15} mol), pressure ejection (double the amount produced by the previous route probably), and forest fires

(3×10^{15} mol). The three previous routes result in a maximum ascent of 5×1015 mol of HNO_3. Tyrrell et al. [33] considered the additions of HNO_3 of 1, 3, and 5×10^{15} mol in their study. The impact is similar to that of H_2SO_4, but considerably smaller.

According to the study by Tyrrell et al. [33], changes in ocean pH are mainly due to the injection of large amounts of H_2SO_4 more than those of HNO_3 and CO_2. The more quickly H_2SO_4 is added to the ocean, the more intense the changes in pH are. Therefore, it is natural that ocean acidification could affect the activity of bacteria, and in particular anammox.

In several studies on anammox, the so-called anammox activity (AA) is used, which is measured according to the production rate of N_2 and is expressed as mmol $N_2\,L^{-1}_{\text{liquid}}\,h^{-1}$, as indicated the equation that follows:

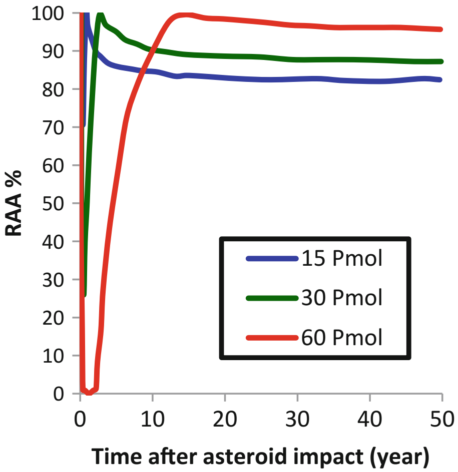
$$AA = \frac{\Delta N_2}{\Delta t} \tag{14}$$

The inhibition was expressed as relative activity of anammox in percent (RAA) by:

$$RAA = \frac{AA_{\text{inhibitor}}}{AA_{\text{reference}}} \times 100 \tag{15}$$

In the above expression, $AA_{\text{reference}}$ is the value of AA to optimal pH and $AA_{\text{inhibitor}}$ is the value of AA at different pH [13]. The effect on RAA associated with the changes of the pH of the ocean is shown in Fig. 3. Inhibition is larger the higher the change in the pH of the ocean and lasts longer in time as the addition of H_2SO_4 is greater.

Fig. 3 Effect of pH on relative activity of anammox (RAA) on suspended culture of anammox. Time e-folding of 6 months



It is to be expected that with rapid additions (smaller timescale), the inhibitory effects on anammox are more abrupt, probably for all H_2SO_4 inputs, but this would be the subject of future work.

The impact of a comet and intense magmatic events/volcanism can generate similar effects on anammox bacteria (and in general, on other chemosynthetic species). These catastrophic events not only affect photosynthetic life as seen in other works, but also the chemosynthetic biota and therefore the habitability of ecosystems and the planet in general.

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